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Provided are biological science laboratory experiments which have been developed and used as a part of an experimental one year undergraduate course in general science for non-science majors. The experiments are directed at the student understanding the balance of chemical and physical processes which must be maintained in order to sustain life, and the importance of our society supporting further research and technological development in the biological sciences. The activities begin with cells and progress through more complex organizational levels. Genetic and hereditary features that characterize living organisms are emphasized. Provided for each experiment are background information, a problem statement, a materials list, laboratory procedures, and questions. (RS)

EXPERIMENTAL VERSION

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LABORATORY MANUAL

Biological Science

E 007169

COOPERATIVE CURRICULUM SOURCE PROJECT

EDO 30575

C.G.S.P. Staff

Biological Science

**Clark
Morehouse
Morris Brown
and Spelman Colleges**

Atlanta, Ga.

PREFACE

For liberal arts majors the biological sciences can be most interesting. No matter what field of endeavor we enter, the study of life is beneficial for all. It is very natural for man to be interested in life and living processes in order to better understand his own body, to be able to alleviate or prevent sickness and ill health, and to better control the factors essential to survival itself since all of our food and most of our clothing and shelter items are ultimately obtained from other life forms. The study of medicine is one of the oldest sciences and was developed because of an urgent need to gain knowledge in this field in order to preserve life and to maintain a state of health. What we today call biology grew out of this broad and ancient concern with medicine.

There is every evidence that man is in all respects a part of the universe in which he lives as evidenced by the fact that in his body are found numerous and diverse mechanisms and systems which are patterned along the lines of the physical, chemical, and mechanical principles that effectively operate on Earth in the non-organic world and extend to the remote regions of the fixed stars. An explication of these areas alone would be a wonderful source of intellectual inquiry, but in the case of living forms and processes we have a whole area of concern which is of a very special kind. The special character of this concern is denoted when we talk about organic processes, organic chemistry and the like when compared to the very different character of the inorganic world and its characteristic processes. The differences are so radical that some scholars have thought it necessary to invoke a special principle to account for living beings and their mode of existence and life processes. But the daily and rapid advances, discoveries, and insights now coming to pass with great frequency have so far fully confirmed the point of view that living processes can and will be explained by the same kind of resources and principles and by the same kind of techniques that have characterized the development and success of the physical sciences. The end is not yet apparent however, and although great progress has been made in biology the future may well contain developments as startling as any that have come to pass so far in any of the sciences.

The following group of experiments has been written and put together by the staff of the Cooperative General Science Project with the hope that the liberal arts majors who take this course will come to understand the delicate balance of chemical and physical processes which nature must maintain in order to sustain life, and to appreciate the importance of our society supporting the research and technology necessary for the further development and implementation of our growing knowledge in this area. An attempt has been made to present the material in as logical and as meaningful a manner as is possible by beginning with a study of cells and then advancing into a study of tissues, organs, and then organisms. Special emphasis has been placed on genetics and the hereditary features that

characterize living organisms. An effort has also been made to relate the materials covered in these experiments and in the lectures out of which these experiments grow to the past experiences of the students. Since it is realized that liberal arts majors will not, in general, be following scientific careers in research and technology, we have attempted to utilize simple but interesting experiments to illustrate basic principles. Learning is easier when the individual can associate the task at hand with previous experience. When the student can project what he is learning into a future useful context he derives additional learning incentive from this fact. These laboratory experiments have been made into an integral part of the course and are designed to complement the lectures by physically involving the students in experiences which illustrate the concepts and principles discussed in the classrooms.

It is hoped that this course in biology and the experiments contained in this manual will together provide the student with a foundation for genuine intellectual adventure, and it is the sincere desire of the staff who developed this manual that its use will be the beginning of a lifetime of enjoyment that is concerned with observing, wondering, and probing into those aspects of the world of life in which we have our beginning and in which we participate as an integral and significant part along with a host of other living forms.

ACKNOWLEDGEMENTS

This experimental version of Biological Science laboratory exercises represents the efforts of many individuals on the staff of the Cooperative General Science Project. While it is realized that some of the staff members contributed more to the development of this work than others, it becomes difficult to give special acknowledgement to a few individuals without slighting others. I wish to express my appreciation to each member of the staff for the interest they have exhibited in this work and the efforts they have expended in its development.

I would like to express special thanks to the United States Office of Education for the financial support of this project. It has contributed greatly to the improvement of our academic program and it is hoped that our efforts in producing this material will be of benefit to other educational institutions.

I am grateful, also, to the administrations of the four participating colleges, to Presidents Vivian Henderson, Hugh Gloster, John Middleton, and Albert Manley for their foresight in establishing this program. We owe special thanks to the various chairmen of the departments who encouraged this program and to deans of the various colleges for their cooperation.

July 30, 1968

Atlanta, Georgia
USA

O. P. PURI
DIRECTOR

TABLE OF CONTENTS

	Page
Preface -----	iii
Acknowledgements -----	v
Instructions -----	1
Exercise	
1 Science - What Is It -----	5
2 Inductive and Deductive Reasoning -----	6
3 The Microscope: Its Use and Care -----	8
4 Diffusion and Osmosis -----	10
5 Cells -----	12
6 Mitosis -----	15
7 Enzymes -----	17
8 Animal Tissues -----	20
9 Blood -----	21
10 Spinal Reflexes -----	23
11 Dissection of Living Frog -----	25
12 Genetics -----	29
13 Plants -----	36
14 The Study of an Ecosystem -----	37
15 Embryonic Development -----	38
16 The Nervous System -----	42
17 The Circulatory System -----	46
Personnel -----	51

LABORATORY INVESTIGATIONS IN BIOLOGICAL SCIENCE

Introduction to the Laboratory

The laboratory is the workshop of the biologists. Investigations within the laboratory, through first hand observations and study of living organisms, have produced the data and information that have been compiled into textbooks. Lectures and textbooks, helpful as they are and indispensable to the study of biology as they may be, provide only second hand information. Real knowledge comes primarily through individual efforts in the laboratory.

A student should approach the laboratory period with anticipation of adventure and a keen curiosity to observe and study living organisms directly. He should learn to work independently and not rely on others for assistance. The significance of each assignment should be recognized and its bearing on the subject under consideration appreciated. The laboratory exercises are correlated with the classroom discussions and should be so related in the student's thinking. Before beginning an exercise, the student should carefully read the directions and then use them as a guide in his study. However, the successful student will not be limited by the directions but will exceed them and launch out on his own explorations and investigations. The extent to which each student progresses in the laboratory will be determined by his curiosity, his ability, and his desire to see things and to interpret them for himself.

AIDS TO LABORATORY STUDY

The objective of laboratory work is to study organisms first hand, not to make a set of drawings or complete a notebook for critical appraisal by the instructor. Drawings are an aid to learning, a means of observing and interpreting that which has been seen. Elaborate drawings are not required and should not be attempted. However, accurateness and neatness are two requisites. Every line should represent a structure. Outlines should be continuous and distinct. Shading is unnecessary and should be avoided. Drawings should be large enough to show necessary details. A tendency of beginners is to make drawings too small. The instructor will explain the acceptable practice of spacing drawings and the method of labeling.

Notes should be taken on observations and features not brought out by the drawings. Answers to questions in the instructions should be thought and written out. Any interpretations or conclusions resulting from your study should be recorded for your future reference. Notes should be made while studying the material, not afterward.

SUPPLIES

Each student will need to supply himself with white, unruled drawing paper, $8\frac{1}{2} \times 11$ inches, for drawing; some theme paper for recording observations, discussions and laboratory reports; a medium hard (4H) drawing pencil, an eraser; a kit of dissection tools; a notebook folder or envelope to contain his drawing, notes and reports. Other materials will be provided by the Department.

RESOURCE BOOKS

The resource books on the shelf in the General Science Office as well as those in the Science Library are for your use. Use them. Do not take these books from the laboratory.

REPORTS

Each student will turn in to the instructor, at the end of the laboratory period or not later than the next laboratory period, a summary report. That the report should be neat, contain clear, grammatically correct English and correctly spelled words, be typed or clearly written in ink on one side of the paper should be obvious. Orienting your report around the following headings should facilitate your work. Do not merely repeat what is in your laboratory directions.

- A. Purpose: Indicate here why the exercise or experiment was done. What were its objectives?
- B. Methods: Describe the procedures followed and any special apparatus or techniques used.
- C. Results: Present the data and results obtained. Very often these data can be given in tabular form; and writing of long, involved paragraphs can thus be avoided. Do not attempt to interpret the data in this section.
- D. Discussion and Conclusion: In this section try to determine the meaning of the data you have presented in the previous section. What answers or conclusions can you find regarding the objectives of the exercise? Why or why not? Remember, negative results may be just as valid as positive ones.
- E. Summary: In single, numbered sentences list the purpose and findings or conclusions of the exercise.

ABSENCE FROM THE LABORATORY

Any exercise that is missed due to absence must be made up during the week in which the absence occurred. Make-up work may be done in any of the regularly scheduled laboratory periods. Consult your instructor regarding make-up.

CLEANING UP

Leave the laboratory as you find it, or better still, as you wish you had found it. Wash any used glassware and other equipment with detergent or other cleanser, using brushes when needed. Then thoroughly rinse several times so that no residue remains. Carelessness in rinsing may spoil a later experiment.

PREPARATION OF LABORATORY DRAWINGS

I. Materials Required:

- A. 4-H drawing pencils
- B. Erasers
- C. Ruler graduated in inches and millimeters
- D. Special biology drawing paper

II. Format: Carefully study the sample of a typical plate found at the end of this exercise and note the following:

- A. Plate number and title - every page has a different plate number in Roman numerals, and each plate has a title in capital letters. These should be centered near the top of the page.
- B. Figure numbers - These are arabic numbers and are not continuous from plate to plate but begin over again with each plate.
- C. Labels - These are usually placed to the right of the drawing and printed in lower case letters. All label lines should be parallel to the bottom of the page approximately equidistant from each other, and should not cross over one another. The labels should form a vertical column.
- D. Other information - Course number, title and section number should be placed in the lower left hand corner of the page and your name and date should be placed in the lower right hand corner.

III. Remarks:

- A. When selecting a single cell in a tissue for your drawing, show that this cell is surrounded by other cells and is part of the tissue.
- B. Strive for a balanced appearance in your plates. Arrange your drawings and their labels so they are centered.
- C. A 4-H pencil must be used for all drawings, labeling, and titling. Shading or coloring may not be used. Stippling may be used with the consent of your instructor.
- D. Draw what you observe, not what your neighbor is drawing.
- E. Reference may be made to your text or other reference books, and structures which you could not actually observe may be added to your drawings, provided such is indicated in the label.
- F. Neatness is desirable, and there is no substitute for accuracy.

Plate I
THE GENERAL CELL

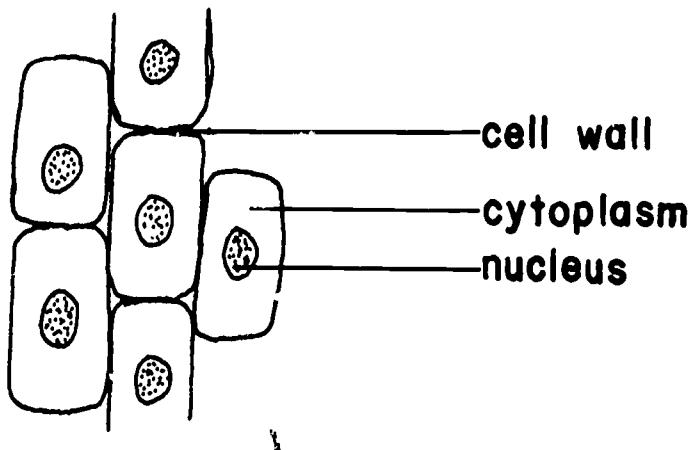


Fig 1 Onion root cells

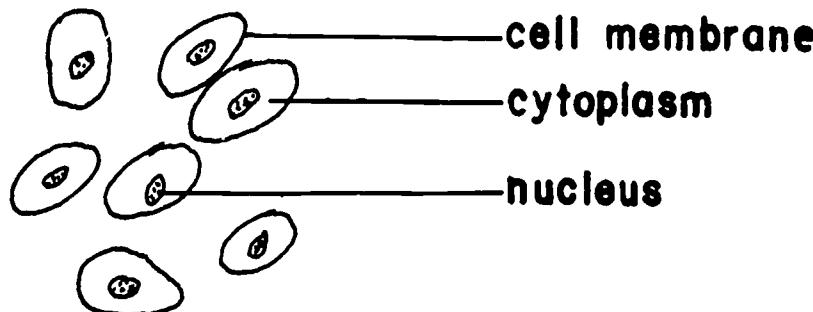


Fig 2 Frog red blood cells

CGS Biology 102
Section X

John Doe
Sept 1, 1968

EXERCISE I

SCIENCE: WHAT IS IT

Introduction: Science is often defined as a body of knowledge, but it is more than that. It is a method of discovery, a means of problem solving. It is the stating of a problem and the seeking of information in various ways to solve that problem. It is, in fact, a way of life.

Problem: To ascertain the contents of a sealed box.

Materials: A sealed box containing three to five objects.

Procedure: You have been given a sealed box containing several objects. In order to ascertain its contents, you may use any approach short of mutilation and/or destruction of the box. You may if you wish make a small hole one-eighth inch or less in diameter in one end and on one side. At various times, you may wish to hypothesize as to the contents of the box. Record all hypotheses and the observations you have made to support them. In fact, record all tests and observations made.

EXERCISE II

INDUCTIVE AND DEDUCTIVE REASONING

Introduction: What you have done in a way in the previous exercise was to utilize the scientific approach to problem solving. This exercise is designed as a further and more ordered application of that method.

Scientific method is characterized by:

- (a) the observation of unrelated facts
- (b) the formation of a hypothesis or hypotheses using some of these facts (inductive reasoning)
- (c) testing the hypotheses
- (d) predicting future occurrences or undiscovered relationships based on the hypotheses (deductive reasoning).
- (e) testing the validity of the predictions.

Do not think because of the above list that the scientific method is a mechanical approach to problem solving. It is not, but rather it is a means of logical thinking based on fact.

Inductive reasoning is a method of reasoning in which generalizations are realized from facts. Deductive reasoning, however, starts with these generalizations and allows predictions of heretofore unknown facts or relationships.

To demonstrate the principles of inductive and deductive reasoning, you will be working with bottles of various types.

Problem: To classify various bottles, to produce identification key, and to identify bottles using a key.

Materials: Fifteen bottles of various types and a marking pencil.

Procedure: The first portion of this exercise is to be done with a partner. First, number all the bottles. Now, by observing characteristics of the bottles, arrange them into categories which show unique characteristics that are common to all the members of that group. Use characteristics such as material they are made of, color, shape and type of top. For example: observation shows that all the bottles are made of one of two substances, therefore the first separation should be into these two categories (this is analogous to the kingdoms of living organisms).

1. Bottles made of plastic
2. Bottles made of glass

Using the two categories above, further subdivide them again using observed characteristics. (this classification is analogous to the phylum level of living organisms). For example:

1. Plastic bottles made of clear plastic.

Further subdivide the above categories still using observed characteristics (this would be analogous to the class level of living organisms). For example:

1. Clear plastic bottles with screw tops.

The second portion of this exercise is to be done individually. In this portion you will construct a branching key by which each single bottle can be identified.

A key is constructed so that there are two statements (called couplets); one of which is true in regards to the item being identified and one is false. At the end of each statement there is a number or letter, or a name which indicates to the user of the key what his next step will be. For example:

- a. Bottle made of plastic
- aa. Bottle made of glass

B
E

In this instance, a person using this key would know that he should proceed to couplet B if the bottle he was identifying was made of plastic or to go to couplet E if it was made of glass.

Using the characteristics already observed and any additional characteristics necessary, construct a key that will enable anyone to identify the bottles that have been given to you.

The third portion of this exercise is to be done individually. Exchange the key you have constructed with your partner and use his key to identify several bottles selected at random from those which were given to you. If the key is constructed correctly and if you are using it correctly, you should come out right in every case. However, if this does not occur, analyze together both your procedure and the key and conclude wherein the error lies and make the necessary corrections.

Questions: Part I

1. The construction of the key from the observations would be an example of what kind of reasoning.
2. The identification of the item by using the key would be an example of what kind of reasoning.

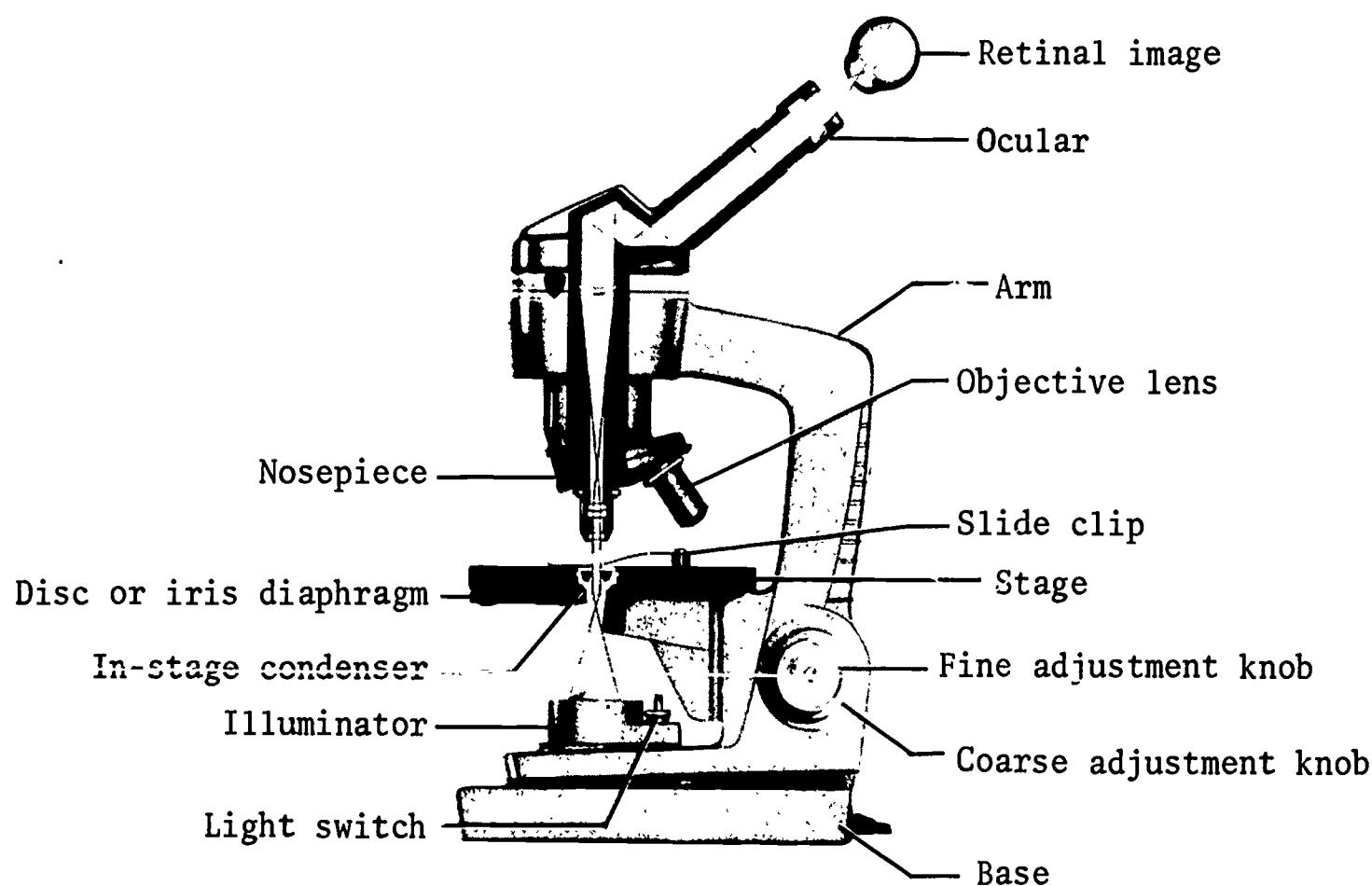
Part II - Tell whether each of the following statements are examples of inductive or deductive reasoning and explain your decision.

1. A person sees a house on fire and looks around for a fire alarm box.
2. Sugar will dissolve in water.
3. A girl refuses to date a certain boy.
4. Rain is forecast for the following day.
5. Leaves are green.

EXERCISE III

THE MICROSCOPE: ITS USE AND CARE

Introduction: The light microscope is one of the major tools of the biologist. It plays an important part in laboratory work as many of the organisms and tissues which are studied are too small to be seen with the naked eye. The proper care and use of this instrument is of utmost importance. Using the diagram of the microscope shown below and the actual instrument in front of you, follow carefully the directions and discussion of the laboratory instructor as he explains the microscope to you.



CAUTIONS

1. Use only lens paper to clean the lenses of the microscope.
2. Never use the high-power objective without a coverslip on the slide.
3. Never try to clean the inside of the eyepiece or the objective. If necessary ask your instructor to handle such problems.
4. Do not tilt the microscope when using a wet mount slide.
5. When the microscope is returned for storage, the condenser should be down, the body tube not extended and the low-power objective in place.

Problem: To learn the proper use and care of the microscope and some of its characteristics by observing various objects.

Materials: Compound microscope, glass slides, coverslips, living microorganisms and prepared slides.

Procedure:

1. Orientation of the image and magnification - Print a very small "e" on a piece of paper. Make it as small as it appears on this page or even smaller. Trim the paper away from the "e" and place it in a drop of water on a glass slide and cover it with a coverslip. Be certain that the "e" is oriented in reading position. Draw the letter "e", natural size in reading position. Place the wet mount on the stage so that the letter "e" is directly below the 10X objective and bring it into focus as previously explained. Now draw it exactly as it appears on low-power. Gently turn the nosepiece so as to bring the high-power objective into the observing position. Focus and draw exactly what you see through this objective.
2. Depth of field - Place a small piece of paper towel on a slide and cover with a small amount of water. Place the slide on the microscope stage and focus until the first fiber or so comes into clear focus. Make a sketch of what you see by using a solid line for those fibers that are in sharp focus and a dotted line for those that are not. Continue to focus upward until the last fiber is in focus. Make at least two more sketches during your focusing, again using solid lines for those fibers in focus and dotted lines for those not in focus.
3. Observation of living material - Make a wet mount slide using material from a culture of micro-organisms.

Practice following these organisms as they dart around the slide until you have mastered the art of moving the slide to keep a single organism under constant observation. Draw some of these organisms.

Questions to be answered:

1. What position does the "e" appear to be in.
2. How much of the "e" can be seen at one time on low power. How much on high power.
3. If a 4X objective forms an image that is four times the size of the original object and the 10X ocular magnifies this image ten times, the image you see would be how many times that of the original object. What magnification would you have with a 10X objective and a 15X ocular; a 43X objective and a 10X ocular.
4. If a micro-organism is moving toward the upper lefthand portion of field of vision, which way would you move the slide in order to keep it in view. (assume that it will continue to move in a straight line).

EXERCISE IV
DIFFUSION & OSMOSIS

Introduction: Some of the fundamental problems of biology deal with the composition, organization and activities of the cell, the basic unit of life. There are, however, many properties of the cell which can be explained and understood in terms of the chemical and physical laws which bear on life processes. Most of the physical phenomena occurring in cells are a result of the fact that molecules are in constant oscillating motion and that the speed of this molecular motion increases with increasing temperature. Molecular motion produces a variety of effects such as Brownian Movement, diffusion, differential membrane permeabilities, and osmosis. Most of these phenomena play vitally important roles in the economy of living systems. Life is as much a result of specific physical reactions as it is a result of chemical reactions. The energy which keeps particles larger than molecules in motion is the kinetic energy of the molecules surrounding them. If it is a bright sunny day, look at the stream of sunlight coming through the windows. Dust particles should be visible as they move rapidly and randomly in the warm air. The movement of these otherwise immobile particles is due to the motion and kinetic energy of the air molecules which surround them. Notice that there is no definite direction to the moving dust particles; it is said to be random motion. Since the dust particles are visible they are larger than the invisible air molecules which surround them and strike the particles on all sides imparting to the dust a motion similar to the motion of the molecules themselves.

Problem: To determine the characteristics of Brownian movement, diffusion and osmosis.

Materials: Test tube, potassium permanganate crystals, test tube rack, thistle tube, syrup, dialysis membrane, ringstand and clamp, beaker, osmometer, Elodea sp., 5% salt solution, microscope, slides, aqueous solution of carmine, and alcohol lamp.

Procedure: Diffusion - Place a crystal of potassium permanganate into a test tube two-thirds full of tap water. Do not shake or stir. Place it in the rack and observe the test tube every half hour during the lab period. Make drawings of your observations.

Osmosis - Hold your finger over the distal end of a tilted thistle tube and pour some syrup into the bulb end of the tube. Cover the bulb end with a dampened piece of dialysis membrane and secure it with a rubber band. Suspend the distal end of the thistle tube by securing it with a clamp on a ringstand so that the membrane is just immersed into a beaker of water. Mark the level of the syrup in the bulb at this time. Observe the apparatus at least three times during the remainder of the lab period and mark the level of the syrup each time. Make drawings of each observation.

Mount a leaf of Elodea sp. in a drop of water on a glass slide and cover with a coverslip. Observe the preparation under high power and note the streaming of the cytoplasm. This movement is called cyclosis. Make a drawing of the cell.

Blot the water away from the leaf with a paper towel and add a drop of 5% salt solution. After five minutes, again observe the leaf under high power and draw what you observe.

Wash the leaf carefully but thoroughly with clean water and then mount the leaf again in water. After five minutes again observe the leaf under high power and draw what you observe.

Brownian movement - Place a drop of an aqueous suspension of carmine on a glass slide and cover it with a coverslip. Observe the slide under high power. Note the erratic, random course of the ceaselessly moving particles. Make a drawing indicating this movement of one or more particles.

Slightly warm the slide by passing it several times through the flame of an alcohol lamp. Be careful not to overheat and thus evaporate the liquid. Again examine it under high power and note the change in the motion.

Questions:

1. What happens in the carmine preparation when it is heated slightly?
2. Does Brownian Movement occur in living as well as non-living situations? Explain.
3. Explain the changes which occur in the tube containing the potassium permanganate in terms of the principle of diffusion.
4. Sugar molecules are much larger than water molecules. In light of the principle of osmosis and your observations of the osmometer, can you prove this statement?
5. What would happen if the bulb of an osmometer were filled with a small amount of distilled water and the membrane end were submerged in a container of salt solution? What would be the direction of the water flow in the above mentioned system?
6. What specific anatomical plant structures are affected by plasmolysis?
7. What causes plants to wilt?
8. What effect would a highly concentrated salt solution have upon a red blood cell? Explain.
9. What is active transport? How does it differ from simple osmosis?
10. What factors affect the permeability of the cell membrane?

EXERCISE V

CELLS

Introduction: The cell is the minimum organization that displays the properties and the processes that we refer to collectively as life. We know life only in the form of cells.

Thus, the cell is often called the basic unit of life. This implies not only that all living organisms (with the exception of viruses) are made up of these microscopic highly organized units but also that all the processes related to life are contained in them. These life processes are performed by minute structures within each cell. These structures are known as organelles.

Although cells vary in a great many respects, they possess certain characteristics in common, such as: cell membrane, cytoplasm, nucleus, nucleolus, nuclear membrane. Most plant cells are contained within a rigid cell wall, composed of cellulose, and may have small green organelles called chloroplasts.

Some living organisms are composed of a single cell; others are multicellular. A multicellular organism may be composed of many different types of cells. A group of similar cells, specialized for a single type of function, is called a tissue.

Problem: To study the structure of plant and animal cells.

Materials: Razor blades, living Zebrina pendula plants, prepared slides of onion root tips, potato tubers, toothpicks, 4.5% calcium chloride solution and an iodine staining solution.

Procedure: Select a leaf from the Zebrina plant that has a purplish undersurface. Peel off some of the epidermis from the undersurface by tearing the leaf diagonally. You should be able to mount a flat piece of epidermis in water with a coverslip and observe the following structures: epidermal cell, cytoplasm, cell wall, nucleus, leucoplasts, guard cells, chloroplasts, stoma, raphides and a purple pigment known as anthocyanin.

Guard cells appear in pairs, which form an oval. The space between them is the stoma; it is usually filled with an air bubble. The guard cell is the only type of cell in the epidermis that has chloroplasts. The chloroplast is a living cytoplasmic organelle which contains the green pigment known as chlorophyll. The cell wall is a non-living jacket around each of these cells and is made of cellulose secreted by the protoplasm of these cells. Look for the nucleus in each of the epidermal cells. Around each nucleus is a ring of colorless bodies known as leucoplasts.

Anthocyanin, the purple pigment, is not confined in special cytoplasmic plastids as is chlorophyll, but is diffused throughout certain cells in the epidermis on the undersurface of the Zebrina leaf.

The cytoplasm has an outer covering, the plasma membrane. This fits so tightly inside the cell wall when the cell is turgid that it cannot be seen unless specially treated. After you have identified all other structures, mount the Zebrina epidermis in calcium chloride solution. The cytoplasm will shrink so that it pulls away from the cell wall, and reveals the plasma membrane.

Plant cells dispose of some excess salts by diffusing them out through the plasma membrane and the cell wall. In this plant, as in many others, certain salts accumulate within the cell. They may be precipitated as crystals. The type of crystal formed in the tissue of this plant looks like a glass needle pointed at each end. It is called a raphide. If eaten uncooked, plants with this type of waste crystal have a very sharp taste which will not diminish until the crystals are dissolved in the mouth; thus, certain plants are inedible unless cooked. Draw several cells from the lower epidermis of Zebrina on your observation sheet and label the following parts (include in your sketch at least one purple cell): epidermal cell, cell wall, raphides, and anthocyanin.

Draw one epidermal cell under high power and label: cell wall, plasma membrane (if seen), cytoplasm, nucleus, and leucoplasts.

Draw one pair of guard cells under high power and label: guard cell, stoma, cell wall, cytoplasm, nucleus, and chloroplast.

Embryonic (meristematic) plant cells have large nuclei. Study the promeristem tissue in the root tip of an onion using a prepared slide. The embryonic tissue is located just back of the root cap. These cells are small and crowded, and have nuclei that are large in proportion to the size of the cells. Some of these nuclei contain chromosomes. Cells that are not dividing show the nuclear structure clearly. There is a nuclear membrane, one or more nucleoli (plural form of nucleolus), and a fine net-like tangle of chromatin threads. These threads shorten and thicken into chromosomes only in the actively dividing cells.

Draw one or two of the promeristem cells on your observation sheet and label the following parts: cell wall, nuclear membrane, chromatin threads, cytoplasm, nucleolus.

With your razor blade, slice a very thin section from a potato tuber. It must be thin enough to be transparent. Rinse it a bit to remove the excess starch grains. Mount it in water with a coverslip. Under the microscope, notice that the cell walls are filled with large starch grains. These potato cells are obviously storage cells. Note the color of the cell wall and the starch grains. Now stain lightly with the iodine staining solution. What color change takes place? Although plants normally store their carbohydrates as starch, non-green plants and animals usually store their carbohydrates as glycogen, which does not take this color when stained with iodine. Draw one of the potato cells on your observation sheet and label the parts you have been able to identify: cell wall and starch grains.

Animal cells resemble plant cells in many respects. An animal cell is nucleated protoplasm without plastids and without a cell wall. Although some animal cells are quite complex, some are simple enough to show the basic cell structures clearly.

To obtain a very simple type of animal cell, we can perform the following painless biopsy with a toothpick. Scrape the inside of your lower lip with a toothpick and mount the material in a drop of water on a slide. Cover and examine under low power. Look for flat transparent extremely thin scales, (cut down the light if necessary). These are squamous epithelial cells which, like all typical protoplasmic structures are transparent and may be difficult to observe. After carefully noting the color of the unstained cells, stain with iodine very lightly under high power, draw one or more of these squamous epithelial cells on your observation sheet and label: plasma membrane, nucleus, cytoplasm, and nucleolus, (if seen).

EXERCISE VI

MITOSIS

Introduction: Among the more striking functions of living systems are the duplication and reproduction of like systems and structures. These characteristically occur at the cellular level of organization. All cells arise from pre-existing cells by a process of cell division, and in ordinary growth this division usually involves the duplication of the set of chromosomes in the nucleus and the separation of these identical sets of chromosomes into two new units, each taking with it a portion of the cytoplasm of the original cell. This process of direct nuclear division may be followed by the division of the cell. Thus, actual division of the cell is always preceded by nuclear division.

Although cell division is a continuous process, biologists, for convenience, recognize several stages, or phases, which grade gradually from one into the other in the living cell undergoing mitosis. Since there are structural differences in plant and animal cells, mitosis varies to some extent in the two kinds of cells, but the process is essentially the same. When studying cross sections of tissues it should be noticed that part of the structure may have been sliced away..

While the number of phases designated by various authors differs, you should be able to identify the following eight stages:

interphase	early anaphase
early prophase	late anaphase
late prophase	telophase
metaphase	daughter cell phase (interphase)

Use the following key to identify the mitotic phases:

A-Chromatin threads and nuclear membrane present	B
AA-Chromosomes present and readily visible	C
B-Cells of normal size	interphase
BB-Two cells occupying the space of one normal cell and separated by a more or less straight line	daughter cell phase
C-Nuclear membrane visible	D
CC-Nuclear membrane not visible	E
D-One nucleus present	early prophase
DD-Two nuclei present	telophase
E-One bundle of chromosomes present	F
EE-Two bundles of chromosomes present	G
F-Chromosomes arranged in a straight line at center of spindle	metaphase
FF-Chromosomes scattered throughout spindle or loosely arranged near center	late prophase
G-The two bundles close together	early telophase
GG-The two bundles widely separated	H
H-Cell membrane not constricted or cell plate not present	late telophase
HH-Cell membrane constricted or cell plate showing	telophase

Problem: To identify the various stages of mitosis and demonstrate their sequence.

Materials: Microscope, prepared slides of Allium and Whitefish embryos

Procedure: Obtain a slide of either Allium or the Whitefish. Find one clear mitotic figure and draw it in the proper place in the sequence of stages noted in the introduction. Continue to select and draw the various stages until all eight have been observed.

Using a slide of the organism not used in the first portion of the exercise, follow the same procedure until all eight phases have been observed and drawn.

Questions:

1. What is the relation of mitosis to the growth of an organism?
2. Does each of the daughter cells contain as many chromosomes as the parent cell?
3. Considering the regions of roots and stems where mitotic divisions occur, what do you infer as to the region where growth occurs in these plant organs and the function of the roots?
4. In single-celled organisms which reproduce by mitosis, would you expect to find variation among the offspring?
5. Are the mechanisms of heredity and genetic variation in any way dependent upon mitosis as you understand it? Think!!!
6. What is the significant consequence of mitosis?
7. How is mitosis similar and different in plant and animal cells?
8. How does the formation of daughter cells in animals compare with that in plants?
9. Assuming that an author made reference to a chromatid, would you think he was referring to the term monad, or referring to the term dyad? Why?
10. If an author made reference to the terms astral rays and asters in describing the protein filaments, would you say he is referring to plant cells, or animal cells?

EXERCISE VII

ENZYMES

Introduction: All living organisms require energy and building materials. Animals and some plants obtain the materials which produce energy by the digestion of food materials. Digestion is a series of chemical reactions resulting in the breakdown of large molecules into smaller ones. Water molecules take part in these reactions, which are thus called hydrolysis (hydro - water; lysis - breakdown). The rate of hydrolysis is increased by enzymes which are produced by cells. Enzymes are organic catalysts (substances which alter the rate of a reaction without themselves being altered) which are specific for the substrate molecule being broken down. The substrates may be proteins, carbohydrates (polysaccharides), or fats (lipids). Proteins are hydrolyzed to polypeptides, then to amino acids. Carbohydrates are hydrolyzed to sugars (di-and monosaccharides). Lipids are hydrolyzed to fatty acids and glycerol.

Hydrolysis of food materials occurs both within the cell (intracellular) and outside of cells (extracellular). Some enzymes are secreted outside of the cells. After the substrate molecules have been broken down, they are small enough to pass through the cell membranes to undergo further reactions inside the cell where there are other enzymes. The release of energy from molecules within the cell is called cellular respiration and involves a series of oxidation-reduction reactions (the loss and gain of electrons or H atoms).

Salivary amylase (ptyalin) catalyzes the hydrolysis of starch (a polysaccharide) into glucose sugar (a monosaccharide). It is an example of a "reducing sugar" because when it is heated with a special solution containing copper, the copper is reduced (the sugar is oxidized), and forms a reddish colored precipitate. The solution you will use is called Benedict's solution. On the other hand, when a drop of iodine is applied to starch, a bluish-black precipitate is formed. Caution should be taken in carrying out the experiments because enzymes are delicate.

Yeast is a unicellular plant which does not contain chlorophyll. Yeast cells carry on cellular respiration in the presence of oxygen (aerobic respiration) and in the absence of oxygen (anaerobic respiration). They contain many kinds of enzymes which catalyze the oxidation of glucose to carbon dioxide and water or alcohol. One such enzyme is a dehydrogenase which catalyzes the transfer of H (hydrogen) atoms from one molecule to another. The dye, Methylene blue, will receive hydrogen atoms from the enzyme and when it does, it turns from blue to colorless (white). The reaction is reversible and the blue color can be restored by shaking the solution in the presence of air in order to oxidize the dye molecules.

Problem: To ascertain the effects of various enzymes under a variety of conditions.

Materials: Green leaves, mortar and pestle, iodine solution, starch solution, beakers, test tubes, Benedict's solution, water bath, starch solution, yeast, glucose solution, methylene blue solution, Smith fermentation tube, mineral oil.

Procedure: Digestive Enzymes - Macerate some green leaves with a mortar and pestle or a blender. (Add some distilled water). Put the macerated leaves into a beaker and pour a small amount into a clean test tube. Add a few drops of iodine. Record your observations on the data sheet. Now pour some starch solution into a test tube and test it with the iodine solution. Record your results.

Now test some of the macerated leaves for sugar by placing it in a test tube with an equal amount of Benedict's solution (2 ml.) Heat the tube in a boiling water bath for 5 minutes, allow the tube to cool and observe the color. Perform the same test with 2 ml. of 5% glucose solution and 2 ml. of Benedict's solution. Has a precipitate formed? Record your results.

Salivary amylase in saliva is an enzyme. Enzymes are made up of polypeptide molecules and are very sensitive to temperature, concentrations, and pH. They are easily inactivated (denatured) by extremes of the three factors.

Gather a clean sample of saliva by first rinsing your mouth with water from the drinking fountain, then depositing about 2 ml. of your saliva into a beaker. Add 20 ml. of distilled water and mix thoroughly. Pour 5 ml. of the diluted salivary amylase into a clean test tube and place it in a boiling water bath for 5 minutes. Meanwhile, set up 4 clean test tubes in a rack and number them. Into each tube, pour 2 ml. of 1% starch solution. Place the first 3 tubes into a water bath of 37°C . Place the 4th in a cold water bath of 10°C . Now remove the test tube which has been boiling for 5 minutes and add some distilled water to restore the volume to 5 ml. When the tube has cooled to body temperature, pour the contents into the 1st tube in the 37 degree water bath. Into the 2nd tube, pour 5 ml. of the untreated salivary amylase in the beaker. Do not add anything to the 3rd tube. To the 4th tube which is in the 10 degree water bath, add 5 ml. of the untreated salivary amylase. Now let the tubes stay in the water baths for 5 or 10 minutes. Meanwhile, set up 2 more sets of tubes (number them 1 to 4).

After 5 minutes, pour a small sample of each solution into the correspondingly numbered tubes. Test the first set for starch by applying a drop or two of iodine. After 10 more minutes, pour a sample into each of the second set of tubes, add an equal amount of Benedict's solution to each and place in a boiling water bath. Remove the tubes after 5 minutes and record your observations. Record your results.

Procedure: - Respiratory Enzymes - Make a yeast suspension with 2 grams of Baker's yeast in 18 ml. of warm water in a beaker. Set it aside for 15 minutes and then examine a drop of the yeast suspension in a wet-mount preparation under the microscope. Draw what you see.

After examining the yeast cells, add 10 ml. of 5% glucose to the suspension in the beaker. Put a few drops of Methylene blue into the suspension until it is a pale blue color. Now pour it carefully into a Smith fermentation tube. Add a thin layer of mineral oil to the surface of the suspension in the tube to seal it from the air. Set the tube aside for 15 minutes. When the time is up, pour the remainder of the contents of the beaker into a test tube and compare the color of the dye in the tubes. Record your results.

Questions:

1. Account for the variety of colors in your tests with Benedict's solution.
2. What are some of the factors which characterize the behavior of enzymes.

DATA SHEET

1.	<u>Substrate</u>	<u>Pre-treatment</u>	<u>Temp. (°C.)</u>	<u>Observations (color)</u>	<u>Results</u>
	green leaves	maceration	25	(a) Test with iodine	_____ is present
	1% starch	none	25	(a) Test with iodine	_____ is present
	green leaves	maceration	25	(b) Test with Benedict's	_____ is present
	5% glucose	none	25	(b) Test with Benedict's	_____ is present
2.	<u>Substrate</u>	<u>Enzyme</u>	<u>Pre-treatment</u>	<u>Temp. (°C.)</u>	<u>Observations (color)</u>
	1% starch	amylase	Heat to 100°C.	37	(a) Iodine test
					(b) Benedict's test
	1% starch	amylase	none	37	_____
	1% starch	none	none	37	(a) _____ (b) _____ (a) _____ (b) _____
	1% starch	amylase	none	10	(a) _____ (b) _____
3.	<u>Substrate</u>	<u>Enzyme</u>	<u>Pre-treatment</u>	<u>Observations (color)</u>	<u>Results</u>
	Glucose	dehydrogenase	sealed with oil	_____	
	Glucose	dehydrogenase	not sealed	_____	

EXERCISE VIII

ANIMAL TISSUES

Introduction: Cells not only evolved a division of labor among themselves, but also developed a tendency for those of similar structure and function to group and act together. Such grouping of similar cells are called tissues.

In animals, a particular kind of tissue may be found in various regions of the body where it may or may not perform the same function. There are six major types of tissues in animals: epithelial, supportive, connective, blood, muscular, and nervous. Although tissues are found in all animals exclusive of the protozoa, we shall consider only those found in the vertebrates such as man.

Problem: To familiarize ourselves with various animal tissues.

Materials: Microscope and prepared slides of various tissues.

Procedure: Using the slides provided, study and draw some representative cells of the following types:

- A. Epithelial
 - 1. squamous
 - 2. cubodial
 - 3. columnar
 - 4. secretory
- B. Supportive
 - 1. cartilage
 - 2. bone
- C. Connective
 - 1. tendon
 - 2. ligament
- D. Muscular
 - 1. smooth
 - 2. striated
 - 3. cardiac
- E. Nervous

Questions:

1. Compare the types of epithelial tissues.
2. Compare the types of supportive tissues.
3. Compare the types of connective tissues.
4. Compare the types of muscular tissues.

EXERCISE IX

BLOOD

Introduction: Blood, the fluid portion of the circulatory system, is composed of the formed elements, the erythrocytes or red blood cells (RBC), the leucocytes or white blood cells (WBC), the platelets, which are fragments of cells that play a role in blood clotting, and the liquid portion, the plasma. In the plasma are the various chemical substances carried in the circulatory system, i.e. nutrients and waste materials, dissolved gases, salts, hormones, proteins, etc.

In humans, and most mammals, the mature RBCs have no nuclei and are shaped as biconcave discs. In the other chordates, the mature RBCs are oval shaped and contain nuclei. The average adult human has 5 to 6 liters of whole blood and there are between 4.5 to 6 million RBCs present per cubic millimeter of blood. Since the mammalian RBC has no nucleus, it can not grow, reproduce or repair itself, so when it wears out, it is replaced by new RBCs produced in the red bone marrow. The color of the blood is due to the hemoglobin, the respiratory pigment, carried in the RBCs.

The leucocytes are divided into two groups, the agranulocytes, which have a single, non-lobed nucleus and contain no granules in the cytoplasm, and the granulocytes, which have a lobed nucleus, consisting of 2 to 5 lobes and there are granules in the cytoplasm. The agranulocytes are the lymphocytes and the monocytes and the granulocytes include the neutrophils, eosinophils and the basophils. The total leucocytes number between 6 to 8 thousand cells per cubic millimeter of blood and there are 50-70% neutrophils, 20-25% lymphocytes, 5-8% monocytes, 1-5% eosinophils and 1% or less basophils present in the total number of human WBCs.

Problem: To identify various blood cells and ascertain the effects of various fluids upon blood cells.

Materials: Sterile hemolets, cotton or cheesecloth, slides, 70% alcohol, distilled water, 0.9% saline solution, 5% NaCl solution and Wrights blood stain.

Procedure: Wipe the tip of one finger with cotton or cloth saturated with alcohol. Allow the alcohol to dry and then prick the finger in one quick motion with a sterile hemolet. The hemolet will enter only far enough to provide an adequate blood supply. Caution, use only a sterile hemolet, never one that has been used by someone else. Discard the first drop of blood, since it may contain tissue debris. Place one drop of blood on a slide. Add 3 drops of distilled water, cover with a coverslip and observe under high power. Add 3 drops of 5% saline solution to a drop of blood placed on another slide, cover and observe. To a drop of blood on a third slide, add 3 drops of .9% saline solution, cover and observe. Make drawings of representative cells on all three slides.

After sterilizing the finger tip, again prick the finger with a hemolet, discard the first drop of blood, and then place a drop of blood near one end of a clean slide which is lying flat on the table. Draw a second slide, held at a 40° (degree) angle into the drop of blood. Allow the blood to flow across the edge of the angled slide, then with a slow and steady motion, move the angled slide along the first slide to spread out the blood thinly and evenly. Allow the smear to air dry. To stain the slide place the blood smear on two parallel glass rods over the sink,

2nd, flood the blood stain with one eyedropper full of Wrights stain and let it stain for 3 minutes; 3rd, add 2 eyedroppers full of distilled water to the stain on the slide and let it stain for another 3 minutes. Rinse off the stain with tap water and let the stained slide dry before observing under high power. In this case no coverslip is needed, although if one wished to keep the slides, a permanent mount should be made. Make drawings of the various types of leucocytes.

The Wrights stain contains 2 dyes - a basic dye, methylene blue, and an acid dye, eosin. The nuclei of all the white blood cells stain with both dyes, as do the platelets and the granules in the cytoplasm of the neutrophils, so they will be purple in color. The granules in the cytoplasm of the basophils stain a deep blue and the cytoplasmic granules of the eosinophils stain a deep red.

Aids for the identification of the blood cells and the platelets.

1. Red blood cells - no staining, since they contain no nuclei or granules in the cytoplasm.
2. Lymphocytes - a purple stained, non-lobed nucleus that fills up most of the cell, surrounded by a small amount of clear cytoplasm.
3. Monocytes - similar to the lymphocyte, except that the cell is larger and there is more cytoplasm surrounding the nucleus.
4. Neutrophil - a purple stained, lobed nucleus, with small purple granules in the cytoplasm.
5. Eosinophils - a purple stained, lobed nucleus, with reddish granules in the cytoplasm.
6. Basophils - a purple stained, lobed nucleus, with bluish granules in the cytoplasm.
7. Platelets - purplish cell fragments that will occur throughout the stained blood smear.

You should be able to locate and identify under high power the red blood cells, the lymphocytes and monocytes, the neutrophils and the platelets. For conclusive identification of the basophils and eosinophils, an oil immersion lens is necessary. If possible, an eosinophil and a basophil will be available for viewing under the oil immersion lens.

Questions:

1. Describe and explain what happened in each of the three tests with the RBC.
2. Describe the types of leucocytes and explain the function of each.

EXERCISE X

SPINAL REFLEXES

Introduction: Spinal reflexes are like any other nervous activity and follow the same route over the five major component parts: the receptor, the sensory path way, the modulator, the motor path way and the effector. The reflex arc, as it is called, is not dependent upon the brain in order that reactions may occur after stimuli have been sensed. As such then, a reflex is a protective action rather than a learning experience. However, it will also serve as a learning experience because the nerve impulse will travel to the brain as well as through the rest of the reflex arc. A stimulus may elicit a single, simple reaction or it may, if it is strong enough or prolonged, elicit a more elaborate response.

Problem: To examine the reflex responses of a living frog.

Materials: Live frogs (Rana pipiens), 1 preserved frog with the brain and spinal cord exposed by dissection, dissecting tools, pins, wax trays, electrical stimulator, physiological saline, Syracuse dishes, dissecting microscopes, cotton swabs, acetic acid.

Procedure: Touch the frog's eye with a pencil. Using a cotton swab, dab some acetic acid onto the back of the frog. Rinse the acid off with some tap water. Now dab some acid onto the belly while lifting the frog off the table. Rinse off the acid with some water. Record your observations.

Using an electrical stimulator, apply an electrode to the back. Increase the voltage and repeat. Now decrease the voltage and touch the belly of the frog as before. Increase the voltage. Record your observations.

Hold the frog with its head bent down and make the pith with a dissecting needle, or make a cut with a pair of sharp scissors where the instructor demonstrates it. The legs of the frog will extend themselves if the pith is successful.

Now suspend the frog by its lower jaw and perform the following: Using either the electric stimulator or the swab of acetic acid, stimulate the leg. Rinse off the acid. Stimulate the back with the electrode or the acid. Pinch the toe of the frog. Record your observations.

Now lay the frog down in a pan and remove the skin of one leg. Locate the nerve between the muscles of the thigh (sciatic nerve) and cut it. Resuspend the frog as before and pinch the toe. Record your observations.

Ask your instructor to show you how to destroy the spinal cord of the frog. Note what happens to the tongue and the nictitating membrane of the eye.

Begin the exercise on the dissection of the living frog. Note the heart continues to beat. Remove the heart and place it in a Syracuse dish containing physiological saline solution at room temperature. Using a second hand on your watch, count and record the beats per minute.

Place the heart in solutions that have been cooled to different temperatures, count and record the beats per minute. Replace the heart in the original solution. Count and record the beats per minute.

Questions:

1. What are the frog's responses to the chemical stimuli?
2. What are the frog's responses to the electrical stimuli?
3. What effect did the cooler solutions have on the heart beat?
4. Did the heart return to the original number of beats when replaced in the solution at room temperature? If it didn't, can you offer any reason why not?
5. What is the pathway of a spinal reflex?
6. What effect does the destruction of the brain's connection with the spinal cord have on the spinal reflexes?
7. Which stimuli are more effective, the chemical or the electrical?
8. What does increasing the strength of the stimulus do?
9. What effect does cutting the sciatic nerve have on the reflex response of the leg?

EXERCISE XI

DISSECTION OF LIVING FROG

Introduction: The frog is a representative animal, useful in the laboratory as an example of the phylum Chordata, Subphylum Vertebrata, to which both man and the frog belong. The frog is in the class Amphibia, while man is in the class Mammalia. The genus and species names of the frogs we will observe are Rana pipiens (grass frog) and Rana catesbeiana (bull frog), and the genus and species name of man is Homo sapiens. Both the frog and man have many similarities in structure and function, but they differ with respect to special anatomical and physiological adaptations which allow them to operate effectively in their own environment. The many similarities can help one to better understand the mechanisms of the human body.

Useful Terms

<u>anterior</u> -	towards the head region
<u>posterior</u> -	towards the tail region
<u>dorsal</u> -	the back or upper surface
<u>ventral</u> -	the belly or lower surface
<u>lateral</u> -	on or toward the side
<u>medial</u> -	on or toward the middle
<u>proximal</u> -	towards the central region
<u>distal</u> -	towards the extremities of the body
<u>bilateral symmetry</u> -	any animal or part of an animal that can be divided into a right half and a left half so that one half is the mirror image of the other.

NOTE - right and left apply to the animals right and left when viewed from a dorsal position.

Problem: To locate the various structures of the external anatomy, the digestive and the respiratory systems.

Materials: Live frog, dissecting kit, and dissecting pan.

Procedure: Notice the coloration of the dorsal and ventral surfaces of the frog and the tightness of the skin attachment. Record your observations.

Locate the external nares (nostrils). These are the two openings at the anterior border of the upper jaw. Examine the eyes and observe the upper and lower eyelids. There is another inner covering of the eye, the nictitating membrane. In humans there is a vestigial nictitating membrane in the medial margin of the eye. Carefully holding the frog in your hand, approach the eyes with a pencil end. See if you can observe their partial retraction into the head. The disc like structures, posterior to the eyes, are the tympanic membranes, which are the outer walls of the middle ear.

Observe the sacral hump caused by the skeleton. The cloacal opening is dorsal and at the posterior end of the trunk. It is the common exit for the products of the intestine, the kidneys and the genital organs. In humans, the opening for the removal of wastes from the digestive tract is called the anus and there is a different exit for the urinary waste products, the urethra.

Notice the difference between the forelimbs and the hindlimbs in size and shape. The forelimb consists of the upper arm, forearm and hands with digits. How many digits are there? In the male, there is an enlargement at the base of the most medial digit (thumb) called the thumb pad, which is absent in the female. The hindlimb is divided into thigh, shank, and foot with digits. How many digits are there? Observe the webbing between the digits. Label as many of these structures on the figure of the frog provided for you.

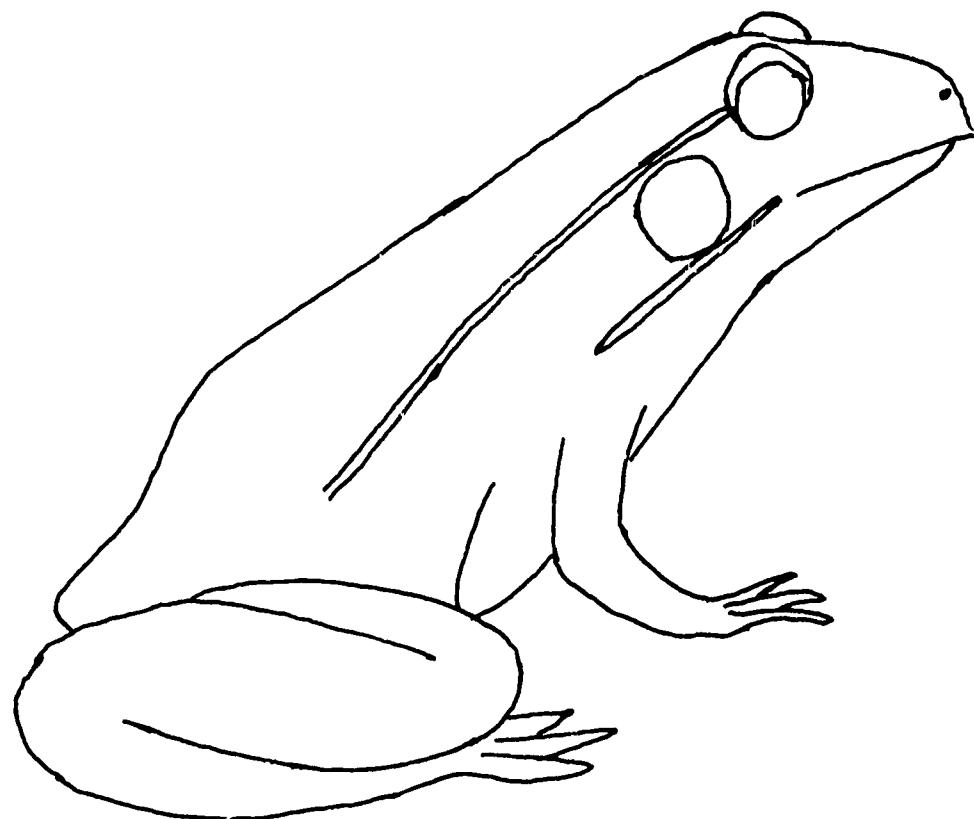
Although the frog is dead, the heart will continue to beat. Peristaltic movements (rhythmic movements) will continue in the digestive tract, and reflex nerve responses may occur.

Place the frog on its back in a dissecting pan and pull back the lower jaw to examine the dorsal area of the buccal cavity (mouth). Observe the maxillary teeth on the upper jaw and the vomerine teeth in the roof of the mouth. Are there any teeth in the lower mouth region? Locate the internal nares; a probe may be inserted carefully through these and it will exit through one of the external nares. Identify the openings of the eustachian tubes in the corners of the mouth. These connect with the middle ear. Examine the tongue and notice where it is attached to the floor of the mouth. Behind the tongue is a slit like opening, the glottis, through which the air passes on its way to the lungs. This is located in the pharynx region. Posterior to the glottis is the opening of the esophagus, through which food enters the digestive tract. Label these parts on the drawing provided.

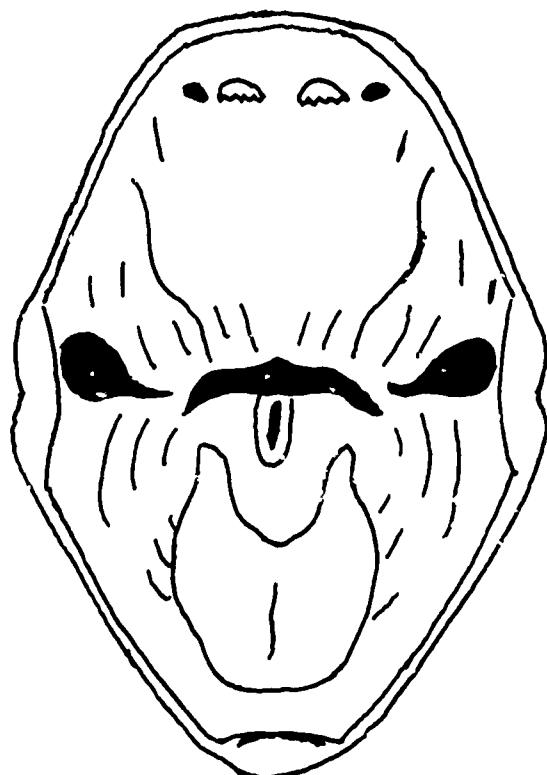
Make a ventral, mid-line incision through the skin from the posterior region of the body to the anterior end of the lower jaw. Make incisions at right angles to the mid line incision at the posterior of the body and just posterior to the arms. Observe the plentiful blood supply of the skin. Now make similar incisions through the musculature of the body wall, taking care to avoid cutting any large vessels. In order to observe the organs in the thoracic (chest) region, carefully cut through the sternum (breastbone) and separate it by pushing it apart with your hands. Pin back the skin and body wall so you can observe the organs in the body cavity. If you have a female, with many eggs in the ovary, you will have to remove the ovaries in order to observe the sections of the digestive tract.

The body cavity (coelom) is lined with a smooth, shining membrane called the peritoneum. This membrane is doubled dorsally and forms the mesenteries which surround and cover the organs which they support. The heart is separated from the rest of the body cavity and lies in the pericardial sac, which is also composed of peritoneum.

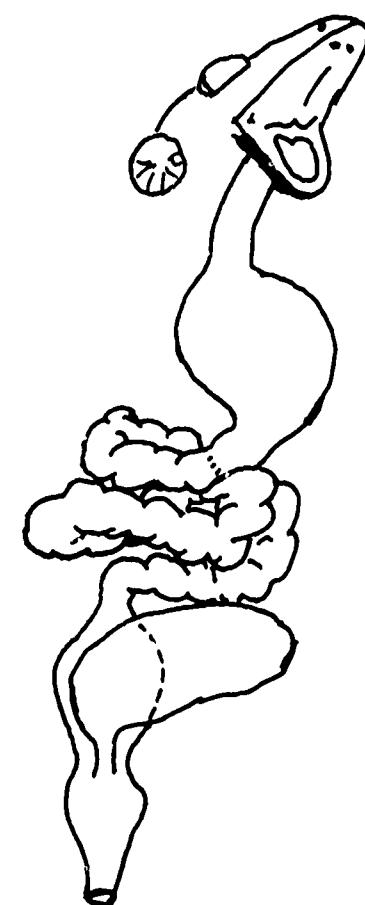
As a means of general orientation, starting at the anterior end of the body cavity, identify the lungs, one on each side. Next locate the conical heart and the blood vessels leading to and from the heart. Posterior to the heart is the liver, composed of three lobes. The stomach is an elongated, whitish structure and the pyloric sphincter is found at the junction between the stomach and the coiled small intestine which connects with the short, large intestine leading into the cloaca. The gall bladder is a small greenish sac found between the lobes of the liver on the right side. The pancreas is located lying in the mesentery between the stomach and first short loop of the small intestine. The spleen, not a part of the digestive tract, is a small, round, dark body located in the mesentery posterior to the stomach. The bi-lobed urinary bladder is located on the ventral side of the cloaca.



Frog



Mouth



Digestive System

Slit the digestive tract from the opening of the esophagus to the posterior portion of the stomach. Observe the inner linings of the esophagus and the stomach and the musculature of the pyloric sphincter between the stomach and the small intestine. Place a small quantity of powdered dye or India ink inside the anterior end of the cut esophagus and observe. A blunt probe may be inserted through the glottis, passing through the larynx leading into the bronchus and then into the lung. There is one bronchus leading to each lung. Label the parts of the digestive tract on the drawing provided and make a drawing of the lungs and heart.

If there is time, have the instructor indicate the location of the kidneys and the adrenal glands; the testes and fat bodies in the male; and the ovaries and fat bodies in the female.

Questions:

1. Of what value would be the difference in coloration of the dorsal and ventral surfaces of the frog?
2. What is the advantage of the web foot to a frog?
3. What purpose would the large blood supply to the skin of the frog serve?
4. What is the difference in the attachment of the frog's tongue to that of the human? Of what value is this type of attachment to the frog?

EXERCISE XII

GENETICS

Introduction: The specific genetic characteristics of an individual, be it plant or animal, are established at fertilization following the union of the male and female chromosome complements, which condition the various characteristics.

Review the processes of mitosis and meiosis and trace through them the fate of the individual genes and pairs of genes. Be able to explain how genes are transmitted from one generation to another.

The test of a working understanding of inheritance is the ability to solve genetics problems. This implies that the meaning of certain terms is clear enough that they can be used correctly:

back cross	homozygous
complete dominance	homozygous recessive
dihybrid cross	hybrid
F ₁ generation	incomplete dominance
F ₂ generation	monohybrid cross
gene	phenotype
genotype	phenotype ratio
heterozygous	zygote

Be sure to check these prior to coming to the laboratory.

It is conventional to represent a dominant character with a capital letter of the name of the character, and to use a lower-case letter for the recessive condition. A gamete is assumed to be haploid, with one gene for each character. Hence, the symbol for a gamete from one of Mendel's pure-line tall pea plants would be T; the gamete from a dwarf plant would be t. The symbols TT, Tt and tt all represent zygotes or the genetic constitution of the plants which grow from these zygotes, i.e. potential parents. The double letter signifies the diploid condition.

A parent which is homozygous, say TT or tt, carries the character in only one condition and consequently can produce only one kind of gamete. A parent which is heterozygous, say Tt, carries on one pair of homologous chromosomes (both a dominant gene for the character on one chromosome and a recessive gene for the character on the other chromosome). In gamete formation, a heterozygous parent will produce two kinds of gametes in equal numbers, 50 percent of them carrying the dominant gene, 50 percent the recessive gene. To predict the offspring from a cross it is necessary to analyze the possible gametes from each parent and consider all possible combinations.

Plants can usually be self-pollinated. Animals seldom can be self-fertilized. This fact modifies slightly the type of problem which may be encountered. Yet the basic principles are the same in both the plant and animal kingdoms. Information from study of genetics in pea plants or fruit flies, corn or guinea pigs, can be applied to human inheritance. To save time, the geneticist tries to learn genetic principles from organisms which have a brief life cycle and

abundant offspring. He may also prefer to follow a dihybrid or trihybrid cross, which takes a few minutes more to analyze, then to test each character separately - every test requiring weeks or months or years.

One further reminder: ratios apply to infinitely large samples, not to limited families of offspring. From knowledge of the parents you can predict what should occur and what may occur among the offspring. From knowledge of progeny you can state what the parents could have been but more than one possibility may remain, with no evidence to indicate definitely one more than another.

Problem: To illustrate some of the concepts and principles of genetics.

Material: PTC paper, hemolet, slides, anti-A and anti-B sera.

Procedure: Do the following exercises.

A. Monohybrid Crosses with Complete dominance

Basic facts: In dogs, "wire hair" is due to a dominant factor, smooth hair to its recessive.

1. How would you represent by symbols a pure-line wire-hair dog?

2. How would you represent a cross between a pure-line wire-hair dog and a smooth-hair dog?

3. How would you show genetic constitution(s) of gametes from the smooth-hair dog, using standard symbolism?

4. What possible combinations of gametes could be obtained in the cross described in 2?

5. What is the genetic symbol for the zygote from the cross in 2?

6. What is (are) the genotype(s) of the offspring from the cross in 2?

7. If two of the F_2 offspring from the cross in 2 were mated, what could you expect as the appearance of their pups?

8. What genotypes would you anticipate finding among the F_2 generations?

9. If a wire-haired dog were mated with a smooth-haired dog and the 8 pups proved to be 3 wired-haired and 5 smooth-haired, what would you conclude about the genotype of the wire-haired parents?

of the smooth-haired parent?

wire-haired offspring?

smooth-haired pups?

Basic facts: In mankind, ability to curl the tongue is found only in individuals who are homozygous recessive for this character. If T is used to represent the dominant condition of this gene, tt will be the individual who can curl the tongue.

1. What kinds of gametes will be formed and what proportions by individuals with the genotypes

TT

Tt

tt

2. Among the offspring of parents whose genotypes are shown, which would you expect to include individuals who could curl the tongue? Proportion?

TT x Tt

3. A man who could not curl his tongue married a woman who could curl hers; they had a child who could curl his tongue. This greatly surprised the husband, for neither of his parents had had that ability. He inquired first of his mother, and she assured him that neither she nor any of her eight brothers and sisters could do such a thing. He inquired from his father, and learned that his father's brother could curl his tongue. The wife then inquired of her parents, and found that neither of them could curl their tongue; they recalled that in both instances their fathers could do so, but not their mothers. Write the probable genotype for the

Child

Mother

Her Mother

Her Father _____

Her Grandfathers _____

Father _____

His Father _____

His Father's Brother _____

His Mother _____

His Mother's Brothers and Sisters _____

His grandparents on his mother's side _____

B. Monohybrid Crosses with Incomplete Dominance

- When a pure-breeding line of red snapdragons was crossed with a pure-breeding line of white snapdragons, all of the seeds produced plants with pink flowers. What flower colors would you expect among the offspring as the following crosses?

Red x Pink _____

Pink x Pink _____

Pink x White _____

C. Dihybrid Crosses with Complete Dominance

Basic facts: In the domestic fowl, black feathers are due to a dominant, and red feathers a recessive of one genetic character. Crested head is dominant to plain head.

- A cock bird known to be pure-line for black feathers and plain head was crossed with a hen known to be pure line for crested head and red feathers. What appearance in these two characters would you expect in the F_1 generation?

- In the F_2 generation? _____

- In a cross between the cock bird and a hen with red feathers and plain head? _____

- In a cross between the hen bird and a cock with red feathers and plain head? _____

D. Human Genetics

1. Each student is to keep a record of the following traits, dominant or recessive, that appear in his phenotype. Record this data on the blackboard. Calculate the percentage of each phenotype and record them on the observation sheet.
 - a. Hold your hands before your face with the palms toward you. Place the two little fingers side by side and press them together. Do they run parallel their entire lengths or do the terminal digits flare out away from each other? Each bone in a finger is termed a phalanx; a flaring phalanx (B) is dominant over a straight phalanx (b).
 - b. Dimples in the cheeks is a dominant trait. The dimples may be more than one per cheek or a dimple may appear in one cheek and not in the other. Dimples (D) is dominant over no dimples (d).
 - c. Darwin's ear point (E) is dominant over no ear point (e).
 - d. Freckles (F) is dominant over no freckles (f).
 - e. Free ear lobe (G) is dominant over attached ear lobe (g).
 - f. Hairs on middle digits on back of fingers (H) is dominant over hairless middle digits (h). Examine your two middle fingers.
 - g. Taster gene for PTC (J) is dominant over non-taster (j)
 - h. Dark hair (M) is dominant over blonde or tawny hair (m).
 - i. Examine the hairline on your forehead. A widow's peak (P) is dominant over a straight or curved hair line on the forehead (p).
 - j. The ability to roll the tongue into a longitudinal U-shaped trough (R) is dominant over the lack of this ability (r).
 - k. Long eyelashes three-eighths of an inch or longer (S) is dominant over short eyelashes (s) less than three-eighths of an inch long.
 - l. Tongue-folding (T) is dominant over the lack of this ability (t). To qualify as a tongue-folder, you must be able to hold the tongue out without bracing it against the teeth and fold the tip back sharply. This is an extremely rare trait. The chances are about 5 to 1 that you will not find this trait in your laboratory section.

Complete the observation sheet. Compare your 12 traits with those of your laboratory partner. Calculate the percentage of agreement. Suppose you had 6 traits alike out of the 12 possible traits: $6/12 \times 100 = 50\%$ agreement. Contact as many class members as you can. Record all agreements. If you have a relative in the class, be sure to compare traits. It would be interesting to see if your relative will have the highest percentage of agreement with you of all the class members.

2. Inheritance of Physiological Character

The ability to taste the chemical compound known as phenylthiocarbamide, commonly called PTC, is inherited. On the average, seven out of ten people, on chewing up a bit of paper treated with PTC, will detect a definite taste while the rest will taste nothing. The individuals who can taste this substance are called tasters, and have the dominant allele T, and thus may be designated as TT. Determine your phenotype for tasting PTC by chewing a piece of filter paper that has been treated.

- a. How many of the class are "tasters"? How many nontasters?
- b. Calculate the number of each and formulate a ratio. Record that data.

- c. Does this ratio approximate the Mendelian 3:1 ratio?

3. Human Blood Groups

The basis for the four blood groups (Groups O, A, B and AB) is the presence of naturally occurring antigens and antibodies. In Groups O, A, B, and AB the factors that determine the antigens and antibodies are inherited, the antigen appearing in the offspring only if present in at least one parent. The two antigens are called A and B and are found on the surface of the erythrocytes. The antibodies are found in the serum and they are known as anti-A and anti-B antibodies. The antigens determining the four blood groups are the result of the expression of three allelic genes (O, A, and B); the latter two apparently being dominant to O. The genotypes AA and AO, and genotypes BB and BO, cannot be distinguished serologically and are classified as phenotypes A and B respectively. Thus, only four phenotypes (A, B, AB, O) can be recognized, although six genotypes occur (OO, AO, AA, BO, BB, AB). The Table shows the distribution of these antigens and antibodies in the four blood group phenotypes.

TABLE 1 - The Human Blood Groups

<u>Phenotype</u>	<u>Genotype</u>	<u>Agglutinogen</u> (Antigen found on surface of red blood cells)	<u>Agglutinin</u> (Antibody found in serum)
O	OO	none	anti-A and anti-B
A	AA or AO	A	anti-B
B	BB or BO	B	anti-A
AB	AB	A and B	none

Each student is to determine the blood group to which he belongs. On a clean microscope slide make 3 circles with a red or black wax pencil. Label one A (for anti-A), one B (for anti-B), and the third C (for control). Place a large drop of blood in each. Add a drop of anti-A (blue) to the blood in the proper circle and a drop of anti-B (yellow) to the other. Stir each drop with a separate needle or toothpick to get a uniform mixture, and after a few minutes note if any reaction has occurred. What is your blood group phenotype.

a. A tabulation of the grouping of the entire class should be made and recorded by the student. What group has the largest percentage?

b. What group has the smallest percentage?

c. A knowledge of the blood groups is of considerable importance in medicine, biology, and in ferensic (legal) medicine dealing with paternity. What types of children are not possible from two AB or two (O) parents?

d. In a case of disputed paternity, the child is group O, the mother group A, and the putative father is AB. What inference can be drawn from this knowledge?

e. What if the putative father were blood group B?

f. Suppose children's identities become mixed up in a hospital nursery. How might blood typing straighten out the matter?

EXERCISE XIII

PLANTS

Introduction: Plants have played a most significant role in the living world as we know it. The fossil record reports that plants were among the first living things to evolve. In becoming adjusted to the primeval environment of the earth, they provided additional conditions for other living things which included an atmosphere in which land animals could survive.

Problem: To learn the parts of an embryonic plant and their importance in the growing process; to determine the factors upon which a plant depends for continuous growth; to test the effect of various wave lengths of light upon the growth of plants; to test physical factors that affect plant growth; and to test the chemical factors that affect plant growth.

Materials: bean seeds, Dixie cups, vermiculite

Procedure: All the beans are to be planted in six inch tall Dixie cups into which have been punctured five holes in the bottom of each cup. One hole is to be punctured in the center of each cup and four additional holes are to be punctured at a radius of one inch from the hole in the center and equidistant from each other. This can be achieved by using the bottom circumference of the Dixie cup as a guide and making four punctures at 90 degree intervals, one inch from the center of the cup.

Unless otherwise indicated, all the beans are to be planted in vermiculite, a prepared medium which has the essential elements for plant growth. Each cup is to be filled with vermiculite to within two inches of its surface after the holes have been punctured. Then, one bean is placed in the center of the cup and four additional beans of the same size (small, medium, or large) are to be placed into each cup with the hilus of each bean pointing to the center of the cup. The beans are to be placed equidistances apart, just as the holes were punctured in the cups. Once the beans are in place, they are to be covered with one-half inch of vermiculite and the preparations are to be watered generously.

For the Student: As the experiments are being performed, additional materials and methods are to be reported. The plants should be watered daily and each cup (experimental plants, and control plants) should receive the same amount of water.

Groups of students will work together and a written report (using the scientific method) is to be submitted. Oral reports will be given to report the findings of each group to the class.

Whatever materials needed will be supplied by the instructors who will aid the students in outlining the procedures for performing each experiment.

EXERCISE XIV

'THE STUDY OF AN ECOSYSTEM'

Introduction: Ecology, or the relationship between plants and animals and their environment is an important phase of biology. For until we understand the many intricate interrelated actions of life, we will have difficulty determining man's place in this world. This exercise is designed to help you realize that there are myriad numbers of organisms in this world and each has his part to play.

Problem: To study a small closed ecosystem and to determine in part the role of its various members.

Materials: Aquarium, microscope, dissecting microscope, identification keys, pipettes, syracuse crystals, slides.

Procedure: Study the aquarium and identify to genus, if possible, all the microscopic organisms in it. (Do not destroy the organisms.) Make sketches of the organisms and list them noting their probable role in the ecosystem.

Using the pipette, forceps, scrapers or any other available means, collect samples of micro-organisms from the surface of the water, from the sides of the aquarium, from the surface of the bottom and from within the soil covering the bottom of the aquarium. Study these under the dissecting and/or regular microscope and identify to genus if possible. Make sketches of the organisms and list them noting their probable role in the ecosystem. Also note the area of the aquarium where the organism was found. By this time you should have a minimum of 20 different organisms.

Make a duplicate of the list of organisms you have identified. Place the number of the aquarium you have studied at the top of the paper and hand it to the lab instructor so that a more complete listing of organisms for that particular aquarium can be compiled. Make an annotated list of the organisms you have found arranged in phylogenetic order.

Questions:

1. What is meant by the term, balanced aquarium?
2. Identify the organisms on your list as to producer, consumer, or reducer.
3. Construct an ecological pyramid to show the relationship between the organisms you have found.
4. Construct a food web based on the information obtained in this exercise.

EXERCISE XV
EMBRYONIC DEVELOPMENT
(Supplemental)

Introduction: An organism is not represented merely by its final adult form - it is all the stages that lead to it. The development of an organism is not a straight-line process; what it is to become is by no means obvious from the start. The tadpole developing into a frog is a classical example. For an organism that eventually develops into a frog, it is strange that it must first become a tadpole. Development in all organisms seems to take a circuitous route. To get a quick view of a not unusual example of development, one only has to notice the development of the frog.

Problem: To observe the development of a chick embryo and identify various structures.

Materials: Hard boiled egg, raw egg, slides of chick embryos

Procedure: Examine a hard boiled half of a chicken egg, (Demonstration) and identify the following: shell, air space at blunt end, membranes under the shell, albumen and yolk. Next examine the contents of a raw egg. On the broken shell of this egg note again the white membranes and the air space. Carefully test the albumen with a probe, and note that some of the egg white is thicker and denser than the remainder. These thick masses are the so called chalazae, two spirally wound strands of albumen, one in the blunt and one in the narrow half of the egg. On top of the yolk mass, examine the blastodisc, a small white spot containing the clear cytoplasm of the egg and the egg nucleus.

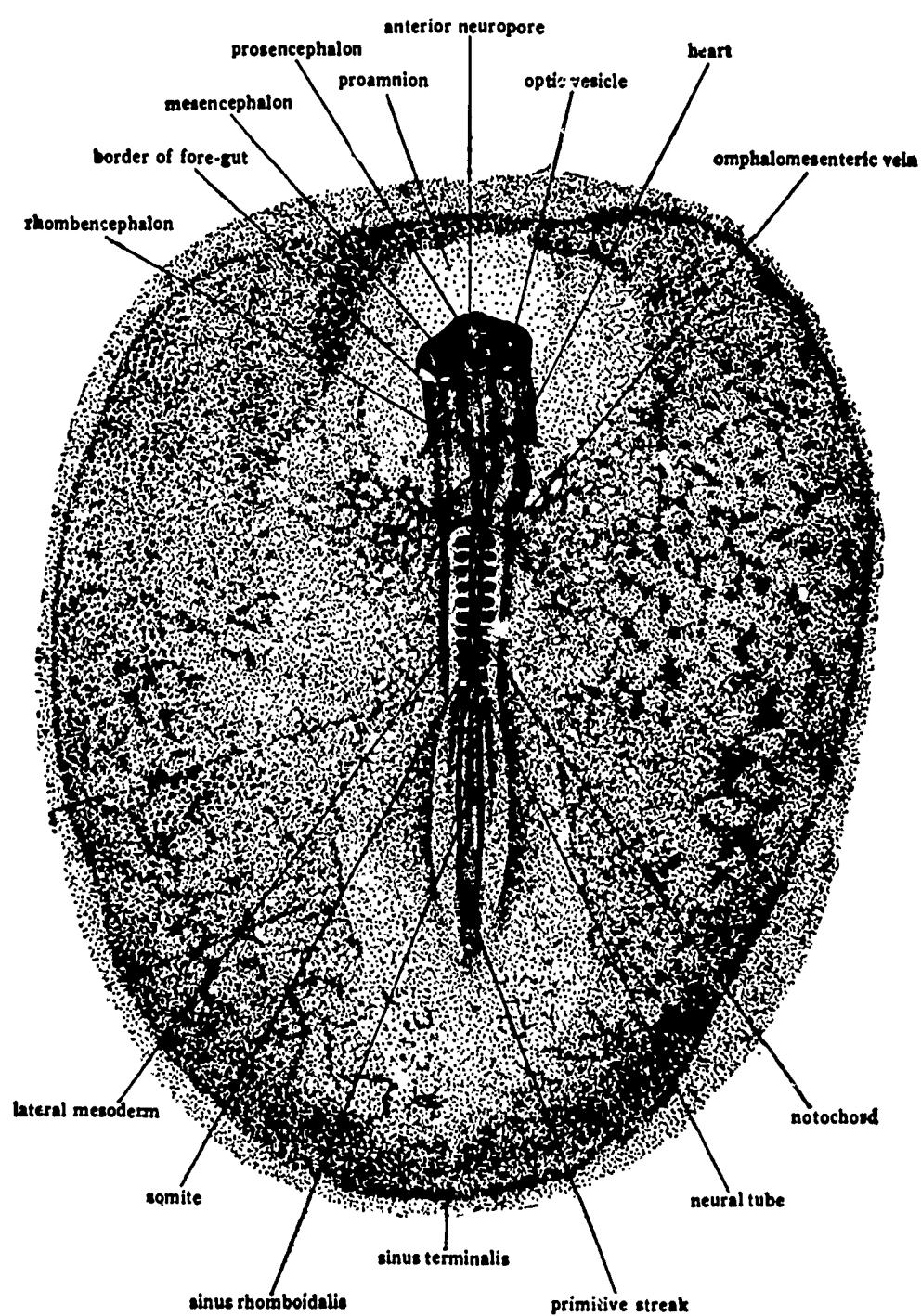
Study the prepared slides of the chick embryo at the following stages, and compare them to the diagrams:

33 hours: An embryo at this stage of incubation will measure 4 mm in total length although a portion of this is represented in a ventral flexion of the head process of the body around the anterior end of the Notochord. Note the large optic vesicles; the constriction between the two portions of the forebrain; the single midbrain vesicle; and the hindbrain.

The heart at this stage is a thin walled S-shaped single, tubular sack which bulges to the right, apparently projecting outside the body. Located the two large vitelline veins which join (sinus venosus) and employ into the posterior part of the atrium. Locate the ventricles and aortic arches anteriorly. Count the number of somites present at this stage.

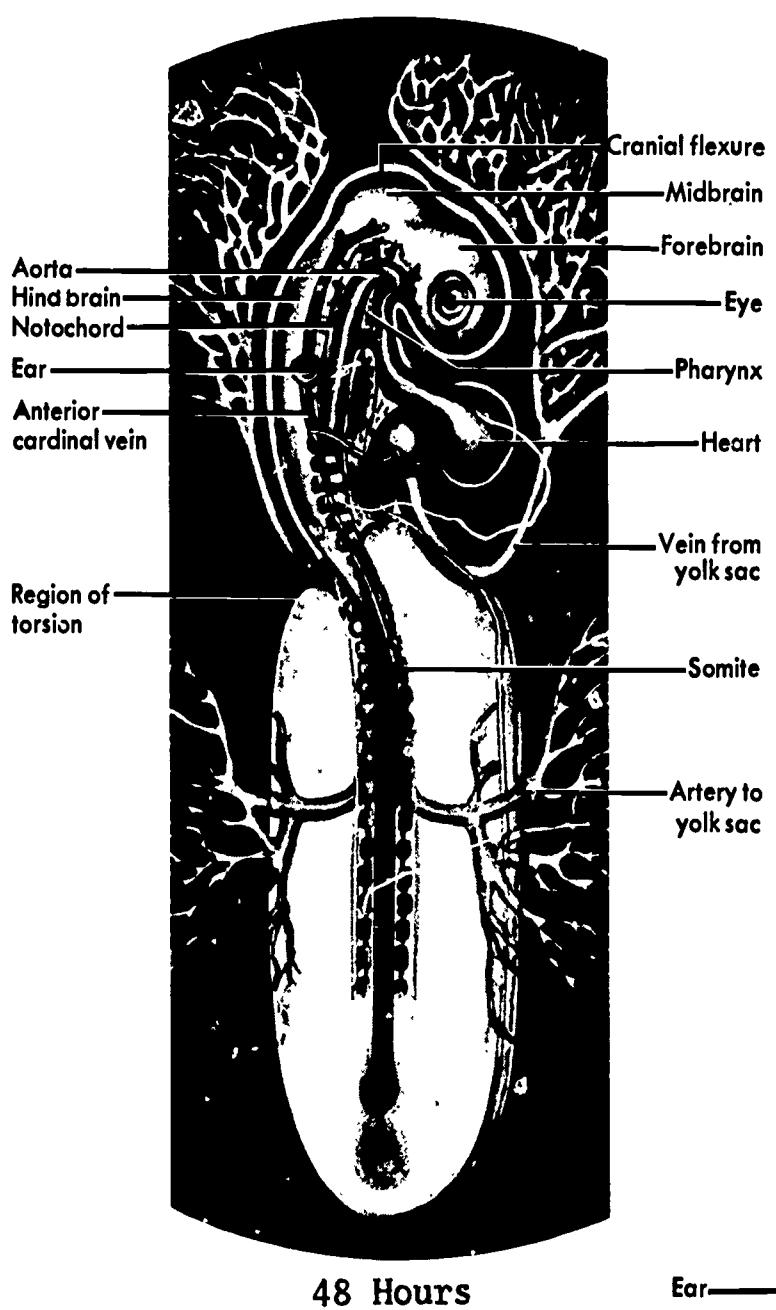
48 hours: two days - An embryo at this stage is already well beyond gastrulation. Due to the rapid growth of the anterior portion (cephalization) of the chick embryo, the 48 hour stage shows both ventral flexion (bending) and a dextral tension (twisting) of the anterior end. The cranial flexure (at mid-brain level) is quite pronounced while the cervical flexure (near junctions of the hind-brain and spinal cord) is just indicated. Identify the following structures: Somites, brain, heart, the vitelline blood vessels (which emerge from about the middle of the trunk and ramify over the yolk within a circular area), and auditory vesicle.

Chicken Embryo

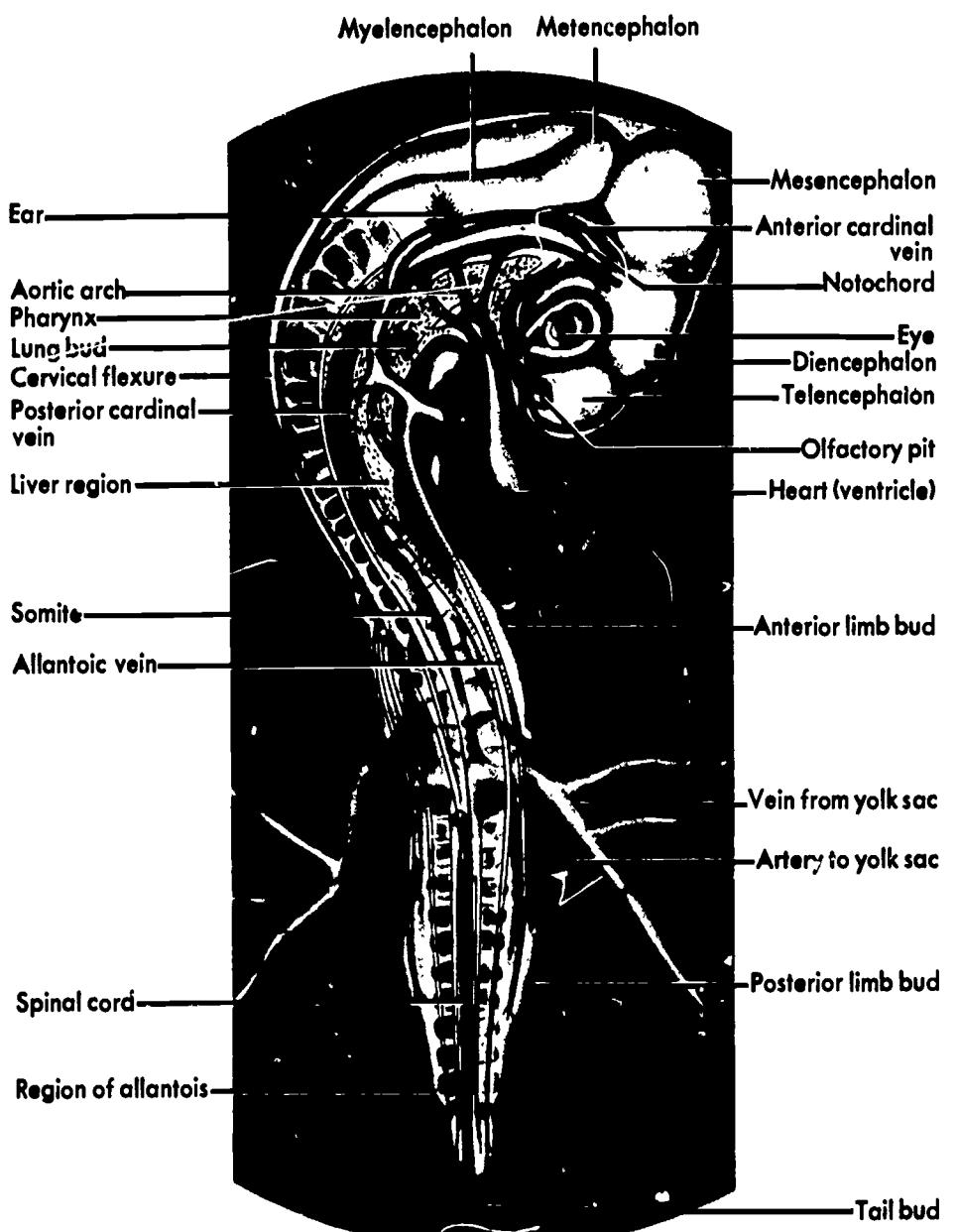


33 Hours

Chicken Embryo



48 Hours



72 Hours

72 hours: three days - At this stage the cranial flexure has bent the head so that the eyes are pointing posteriorly and accentuated cervical flexure carries the myelencephalon and the spinal cord out to the left from the original mid-line of the embryo. The brain shows considerable enlargement with thickenings and thinnings of the walls at various levels. Locate the previously described "landmarks" of the major brain vesicles, the five parts of the brain now clearly delineated. Note eyes and ear vesicles. Identify the head and limb bud (a bulge of tissue which will give rise to the wing on that side). Note changes in circulatory system.

96 hours: four days - The overall length of the 96 hour chick embryo is not significantly greater than that of the 72 hour stage because the pronounced cranial cervical and caudal flexures have compensated for most of the actual cephalo-caudal growth. The main body divisions of head, trunk, and tail are better defined and the appendages, the allantois, a transparent fluid filled sac, and the very large vitalline veins and arteries can easily be located. The wing (fore-limb) buds appear at the level of Somites #17 to #19, while the leg (hind limb) buds appear at the level of Somites #26 and #32, near the tail bud. Locate the five divisions of the brain, the heart, eye and auditory vesicle.

EXERCISE XVI

THE NERVOUS SYSTEM (Supplemental)

Introduction: In order to promote their survival, organisms have the ability to respond to chemical and physical stimuli of the internal and external environment. Highly specialized cells and tissues capable of receiving and interpreting these stimuli (messages) make up the nervous system. In higher forms of life, such as in the case of the vertebrates, the nervous system is an elaborate and very complex organization of cells which carry messages from one part of the body to another. In the nervous system these messages are electrochemical in nature and travel along individual nerve cells between the body receptors and effectors and the central nervous system. The structures which detect stimuli are called receptors. Organs, such as muscle, respond in some physical way to stimuli and are called effectors. The basic morphological unit of the nervous system is the neuron (nerve cell). Nerve cells vary greatly in length and thickness. The typical neuron is composed of three parts: the cell body (containing the nucleus); an axon (nerve fiber); and a dendrite. The axons and dendrites are extensions or processes (sometimes called neurites) of the cell body. Messages are carried toward the cell body via the dendrite (afferent). Axons carry messages away from the cell body (efferent). A nerve is a bundle of neurites. A collection of neuron cell bodies is called a ganglion.

The vertebrate nervous system is composed of three main parts: the central nervous system (CNS) composed of the brain and spinal cord; the peripheral nervous system composed of the spinal nerves which are subdivided according to function; and the autonomic (sympathetic) nervous system which controls the cardiac and visceral muscles.

Problem: To study the parts of the nervous system of the frog.

Materials: Preserved frog and dissecting kit.

Procedure:

I. The Central Nervous System

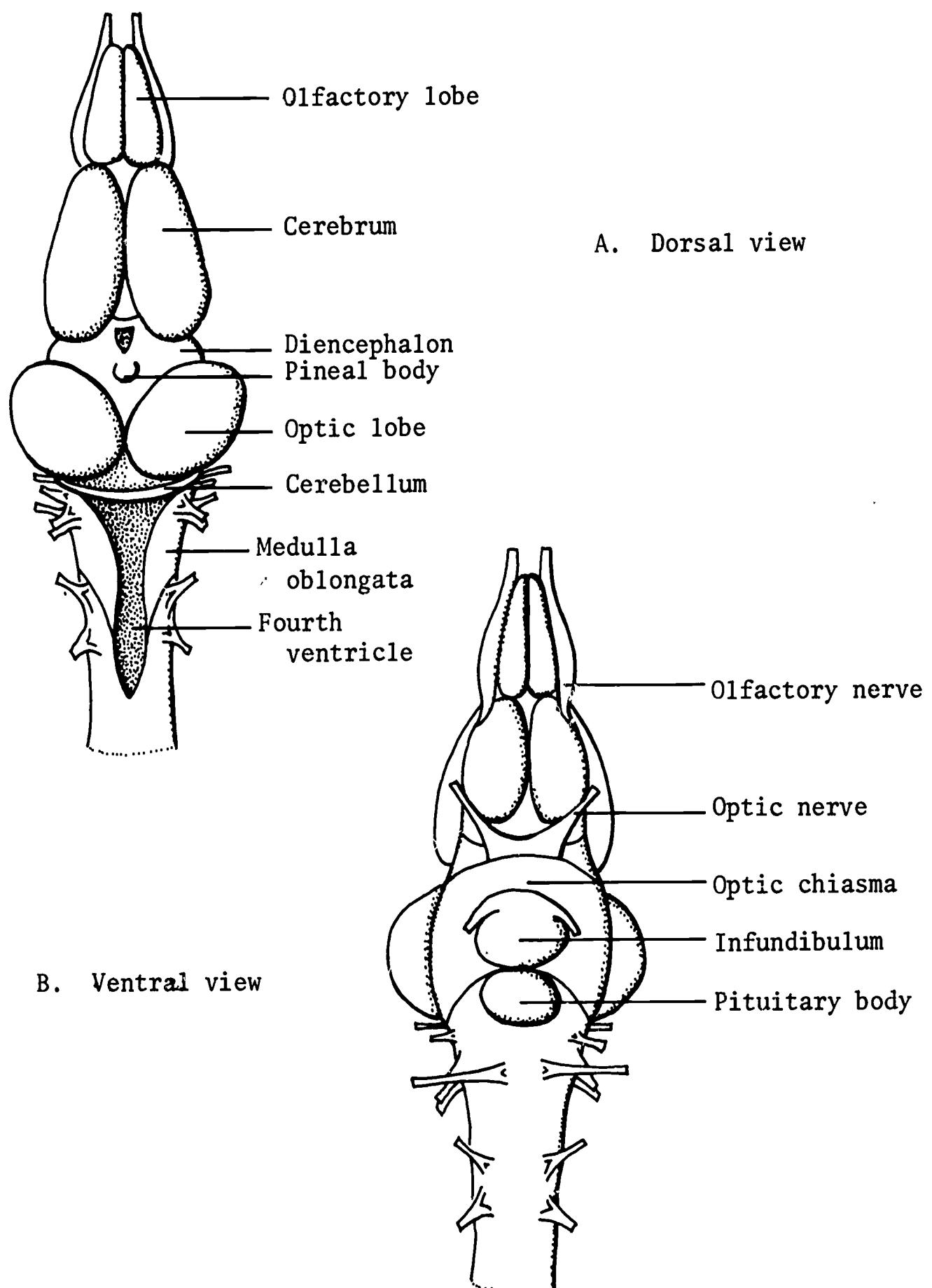
A. Brain and Spinal Cord (Dorsal Aspect)

Beginning at the anterior end of the frog, cut through the skin on the dorsal surface and remove it, exposing the dorsal musculature. Cut through the skull just behind the external nares and carefully chip away the bone of the roof of the skull using scissors and forceps.

CAUTION: Be careful not to injure the underlying structures. If the dissection is performed carefully, the two membranes which comprise the meninges can be identified. The first membrane which lines the cavity of the skull is called the dura mater. A second, much thinner membrane, called the pia mater closely covers the brain itself.

Beginning at the anterior end of the brain, identify the following dorsal structures. (Consult attached diagram for assistance if necessary.)

1. Olfactory lobes are located most anteriorly on the dorsal surface. They give off the olfactory nerves from their anterior ends.



2. Cerebral hemispheres (Cerebrum): Located just posterior to the olfactory lobes, the cerebrum is composed of two relatively large hemispheres. Located just below and behind these hemispheres is a small, depressed region called the diencephalon. It may not be clearly visible in the dorsal view.
3. Optic lobes: The rounded paired structures are located posterior to the diencephalon and make up most of the midbrain.
4. Cerebellum: Located posteriorly to the optic lobes, the cerebellum of the frog is much smaller than in most vertebrate animals and forms a small narrow strip.
5. Medulla oblongata: This broad structure posterior to the cerebellum is covered by a thin vascular membrane and is continuous with the spinal cord posteriorly.
6. Fourth ventricle: This structure is a triangular cavity located to the rear of the medulla and it is continuous with the central canal of the spinal cord.
7. Spinal Cord: The spinal cord extends posteriorly on the dorsal surface.

B. Brain (ventral aspects)

Using great care, remove the brain from the cranial cavity trying not to tear the delicate tissue. Sever the olfactory nerves just anterior to the olfactory lobes and carefully lift the brain. As you proceed, continue to lift the brain as you sever the cranial nerves which emerge from its ventral side.

When the entire brain has been freed, cut across the spinal cord and remove the brain from the cranial cavity. Place it so that the ventral surface can be examined.

Identify the optic chiasma which is the cross-over site of the right and left optic nerves. In the midline just posterior to the optic chiasma you should be able to identify the infundibulum which is the attachment site of the pituitary gland. (The gland is usually torn away in the process of removing the brain.)

II. The Peripheral Nervous System (Cranial and Spinal Nerves)

A. Cranial Nerves: In the frog there are ten cranial nerves. In man, there are twelve, the first ten of which are like those of the frog.

Examine the brain which has just been removed from the cranial cavity. At the most anterior end of the ventral surface are the olfactory nerves which extend anteriorly. Anterior to the optic chiasma and just posterior to the optic lobes are the optic nerves.

B. Spinal Nerves: Carefully remove the viscera (internal organs) from the body cavity taking care not to sever the dorsal aorta. The spinal nerves emerging from the successive vertebrae will be visible. Each nerve is

attached to the spinal cord by two roots. The dorsal root is marked by an enlargement called the dorsal root ganglion which is not visible due to overlying materials. The dorsal root is entirely sensory in function. The ventral root which is entirely motor in function has no ganglion and all nervous impulses travel away from the spinal cord via this root. Identify the following structures.

1. The brachial plexus innervates the muscles of the body wall and is formed by the contributions of the first three spinal nerves.
2. The fourth, fifth and sixth nerves pass laterally to the body wall and form the lumbar plexus.
3. The sciatic plexus is formed by the seventh, eighth and ninth spinal nerves. The largest nerve coming from this plexus is the sciatic nerve, the largest in the body.

III. The Autonomic Nervous System

(DEMONSTRATION) The frog to be used in demonstrating the autonomic nervous system has been placed in a solution of 0.3 - 0.6M nitric acid overnight to make the nerves more visible.

Two delicate thread-like structures, one on either side of the vertebral column, are the main trunks of the sympathetic system and extend from the skull to the last spinal nerve. They lie close to the dorsal aorta, and diverge anteriorly where the systemic arches unite. These may be seen by gently lifting the dorsal aorta. Branches from the main trunks connect with the spinal nerves, one from each nerve, except the eighth and ninth to which there are two and three respectively. White swellings on the main trunk are sympathetic ganglia, one for each connection with a spinal nerve. Branches from the fourth, fifth and sixth ganglia unite to form the splanchnic nerve, which follows the common artery to the digestive organs.

Questions:

1. How does the nervous system interpret changes in the external environment? Give an example.
2. Describe the nature of a nerve impulse and how it is thought to travel along a nerve fiber.
3. How does the neuron differ from the generalized cell type studied previously?
4. How does the body control the function of such spontaneous activities as circulation, respiration and digestion?
5. What behavioral evidence exists to suggest that sensitivity to stimuli is a property of all living things (plant, animal, etc.)?
6. Atropine is a drug which causes acceleration of the heart beat, decrease in the secretion of saliva, and dilation of the iris of the eye. What part of the nervous system does atropine stimulate? Prove your answer.
7. Would you consider the lack of willful control of the autonomic nervous system a fortunate or an unfortunate state of affairs? Why?

EXERCISE XVII

THE CIRCULATORY SYSTEM (Supplemental)

Introduction: The circulatory system of a land dwelling vertebrate is, of necessity, a complex set-up. The blood must be circulated so that oxygenated blood is in one part of the circuit and deoxygenated blood in another. (The means of oxygenation of the blood in the lungs further complicates the job of keeping the two types of blood separated at the heart which is the common organ serving both arteries and veins.) This is solved by the use of valves. However, the frog heart is not as efficient at this since it has but one ventricle, while the birds and mammals have two.

Problem: To study the circulatory system of the frog.

Materials: Preserved frog, dissecting kit

Procedure: Place the frog on its back in the dissecting pan. Use your scissors to make a cut slightly to the left of the midline, and extend the cut from the pectoral girdle to the cloacal opening. Cut through the skin and body wall, being careful not to damage any of the organs within the body cavity. Cut through the sternum (breastbone) to the midpoint of the lower jaw. Make cuts at right angles to the midline at each end, and pin back the flaps. You may need to fasten a rubber band across the back from one foreleg to the other in order to keep them stretched apart.

The blood vessels have been injected with latex, red for arteries and blue for veins. But you should learn to know these vessels by seeing what organs they serve. As you dissect the arteries with forceps and scissors, work on the left side of the heart. Later you will dissect the corresponding veins on the right side. You may have to remove parts of the pectoral girdle after you have separated the muscles in order to see the blood vessels.

A. The Heart and the Arterial System

The heart is covered by a pericardial membrane. The space in which the heart lies is called the pericardial cavity. Remove the pericardial membrane. The posterior portion of the heart is the thick-walled ventricle. A tubular chamber, the conus arteriosus, extends anteriorly from the right side of the ventricle to fork into two vessels, the right and left truncus arteriosus. The conus arteriosus lies across the right and left atrium of the heart. The right atrium is the larger one.

Trace the branches of the left truncus arteriosus. It has three branches or arches.

The first branch goes anteriorly to the head and is the carotid arch. It supplies the brain, the mouth, and adjacent areas. The pulmocutaneous arch curves posteriorly to fork into the cutaneous artery which supplies the skin of the shoulder and back, and the pulmonary artery which supplies the lungs.

The middle arch is the systemic arch. It curves posteriorly around the heart, giving off two branches before it meets its partner from the right side where they join to form the dorsal aorta. One branch of the systemic artery supplies the vertebral column and skull. The other branch, the subclavian artery, extends into the arm.

Now trace the dorsal aorta posteriorly. Just behind the union of the two systemic arches, a large single artery branches off ventrally from the dorsal aorta. It is the coeliacomesenteric artery and it divides into the coeliac artery, which supplies the liver, stomach and pancreas, and the anterior mesenteric artery, which supplies the spleen and the small intestine.

More posteriorly along the dorsal aorta, there are paired arteries branching off to the kidneys, the reproductive organs (gonads) and the fat bodies. These are the urogenital arteries. There is also one artery which goes to the large intestine, the posterior mesenteric artery.

The dorsal aorta now forks into the two common iliac arteries, one into each leg. Trace the main branch of the left iliac artery which lies between the gastrocnemius muscle and the tibiofibula (shinbone). This branch is the sciatic artery.

The branches of all of the arteries divide and subdivide to form the smaller arteries (arterioles) and the capillaries. The blood drains from the capillaries into small veins (venules) and then into the veins.

B. The Venous System

Lift up the heart so that you can dissect the veins on the dorsal side. The sinus venosus is a large, thin-walled triangular sac which receives blood from three large veins and drains into the right atrium of the heart. Two of the three veins are the precaval veins, or anterior vena cavae.

Follow the frog's right one. Three branches come into it: the external jugular vein from the throat, the subclavian vein from the arm, and the innominate vein which is between the external jugular vein and the subclavian vein. The innominate vein receives two branches, the internal jugular vein from the brain, and the lateral subscapular vein from the shoulder.

The subclavian vein receives blood from a large brachial vein from the arm, and a smaller musculocutaneous vein from the body wall.

The third vein entering the sinus venosus is the single postcaval vein or posterior vena cava. It can be seen emerging from the liver. Posteriorly, it receives hepatic veins from the liver, efferent renal veins from the kidneys, and genital veins from the reproductive organs.

The ventral abdominal vein can be seen lying along the midline of the ventral body wall. Anteriorly, it joins a vein which leads into the liver, the hepatic portal vein. Posteriorly, the ventral abdominal vein is divided into the two pelvic veins which receive blood from the large femoral veins of the hind legs.

C. The Portal Systems

Unlike the other veins, which carry blood from the capillaries directly into the heart, the portal veins carry blood from one capillary network to another capillary network.

1. The hepatic portal system. The hepatic portal vein runs parallel to the bile duct and into the liver. It receives blood posteriorly from the gastric veins (from the stomach), the pancreatic vein, the splenic vein, and the intestinal veins. These veins may be found in the mesentery.

The blood of the hepatic portal system originates from the coeliacomesenteric artery which carries it to the intestinal capillaries where the products of digestion are absorbed. From these capillaries, the blood flows into the hepatic portal vein and on into the capillaries of the liver where some nutrient materials are stored and some waste materials released. Going from the liver, via the hepatic veins, the blood then returns to the heart in the postcaval vein.

2. The renal portal system. (This system is not present in mammals.) The right renal portal vein may be found by lifting up the frog's right kidney and looking along the dorsolateral surface. The renal portal vein has branches going into the kidney called afferent renal veins. (Efferent renal veins leave the kidney and empty into the postcaval vein). Posteriorly, the renal portal vein receives blood from two veins. One is the small sciatic vein from the leg. The other is the short segment known as the iliac vein, coming from the femoral vein of the leg. A third vein which enters the renal portal vein is the dorsolumbar vein which drains the dorsal body wall.

Notice that the blood from the hindlegs can return to the heart in two different paths, (1) from the pelvic vein by way of the ventral abdominal vein and the hepatic portal system or (2) from the iliac vein and through the renal portal system.

D. The Heart - Internal Structure

Using your scalpel, start at the posterior tip of the ventricle and partially saw off the front of the heart so as to be able to lift it up ventrally. You should also cut through a portion of the conus arteriosus so that you can see into it.

The larger right atrium is separated from the left atrium by the interatrial septum. The atrioventricular valves are flaps which lie between the atria and the ventricle. These valves prevent backflow of blood into the atria when the ventricle contracts. At the entrance of the sinus venosus into the right atrium there are two dorsal sinoatrial valves. In the conus arteriosus, there are several valves. Proximal to the ventricle are three semilunar valves. Along the dorsal wall of the conus arteriosus is the spiral valve. At the distal end of the spiral valve, two smaller semilunar valves may be seen on the right side.

In the dorsal wall of the left atrium, the entrance of the pulmonary veins may be found.

Questions:

1. Which blood vessels empty into the sinus venosus?
2. From the sinus venosus, where does the blood go?
3. Trace the circulation of the blood from the heart to the lungs and back to the heart, and name in order all the parts involved.
4. What are two other sources of oxygenated blood besides the lungs?
5. Is there mixing of oxygenated blood with oxygen-depleted blood in the heart? Where?
6. From what organ does the hepatic vein receive blood?

7. From what organs does the hepatic portal vein receive blood?
8. Which arteries supply the brain with blood?
9. What is the connection between the renal portal system and the hepatic portal system?
10. What is the difference between an afferent renal artery and an efferent renal artery?

PERSONNEL

ORGANIZATION AND ADMINISTRATION

Dr. O. P. Puri
Project Director

Professor W. P. Thompson
Associate Professor

Dr. V. W. Henderson
President - Clark College

Dr. H. Gloster
President - Morehouse College

Dr. John Middleton
President - Morris Brown College

Dr. Albert Manley
President - Spelman College

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